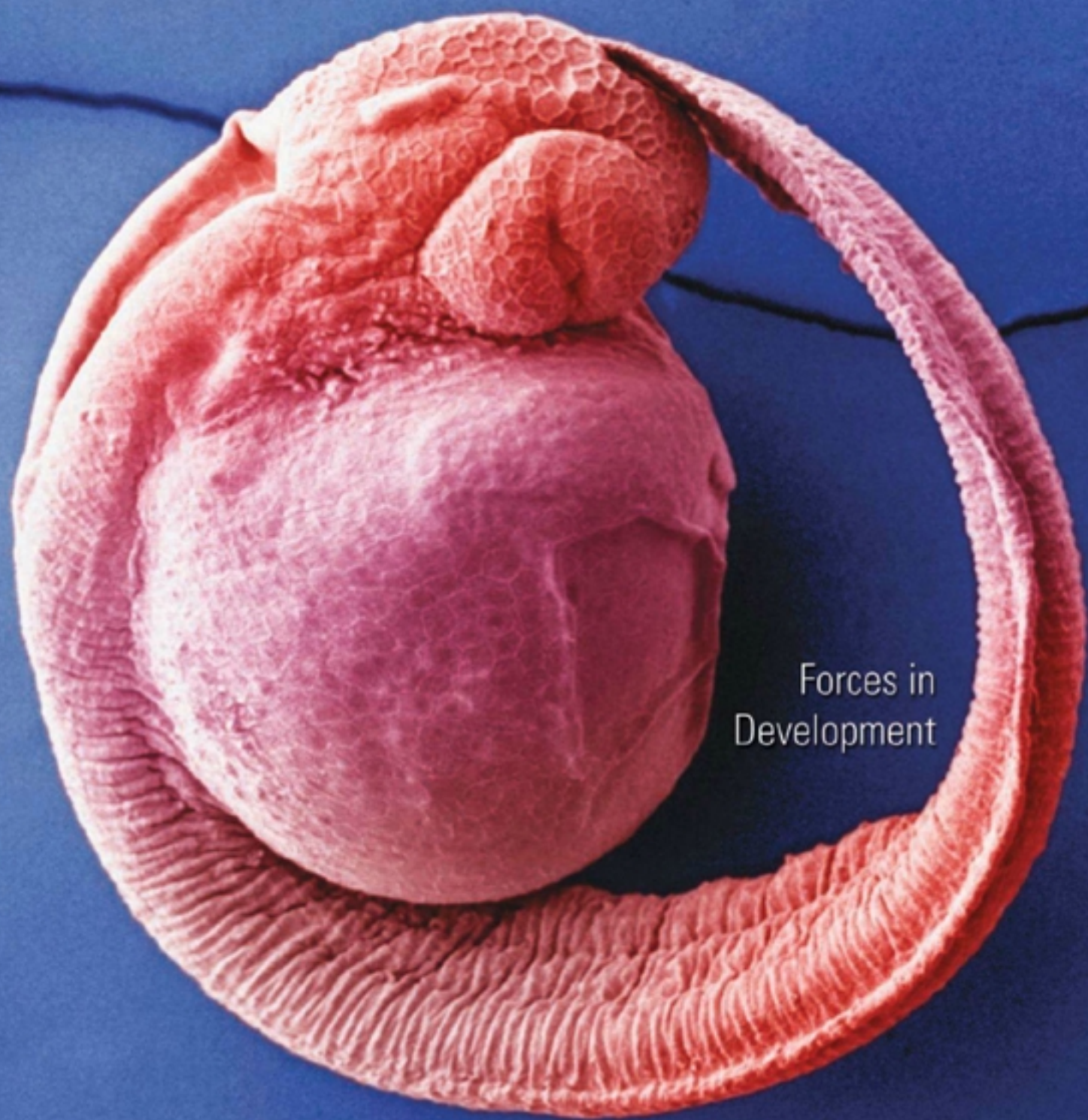


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Science



Forces in
Development

 AAAS

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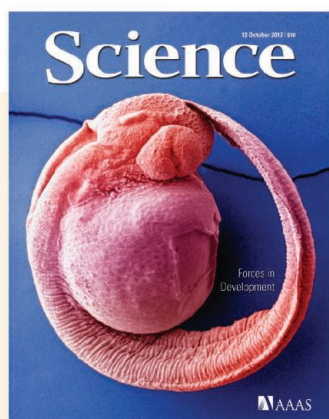
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False-colored scanning electron micrograph of a zebrafish (*Danio rerio*) embryo (~0.7 millimeters across) at a late stage of development. Biophysical studies of model organisms such as zebrafish reveal forces that organize tissue patterns and shape cell layers and physical features (segments, cavities, and folds). The special section beginning on page 209 presents work in which physicists and biologists collaborate to explain the physical mechanisms of animal development.

Image: Dr. Richard Kessel/Visuals Unlimited, Inc.

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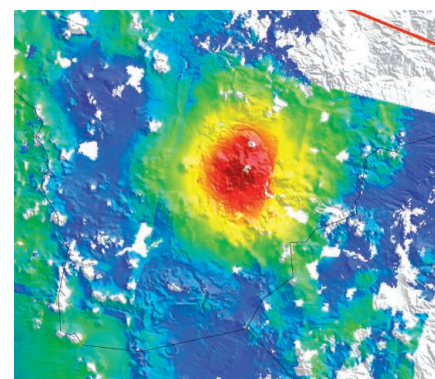
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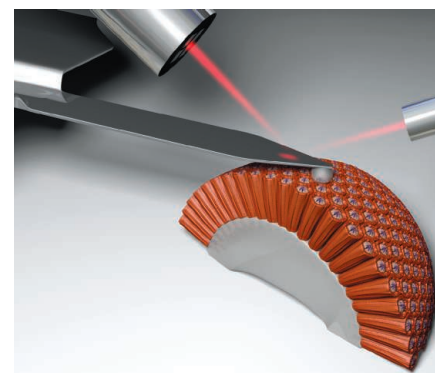
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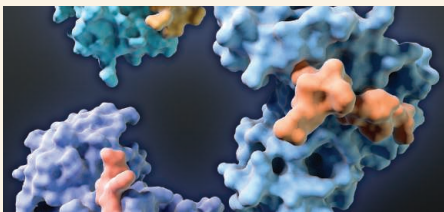


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H. Chennaoui Aoudjehane et al.
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D. E. Cook et al.
10.1126/science.1228746

Tug-of-War in Motor Protein Ensembles Revealed with a Programmable DNA Origami Scaffold
N. D. Derr et al.
10.1126/science.1226734

Flows of Research Manuscripts Among Scientific Journals Reveal Hidden Submission Patterns
V. Calcagno et al.
10.1126/science.1227833

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Financial Costs of Meeting Global Biodiversity Conservation Targets: Current Spending and Unmet Needs
D. P. McCarthy et al.
10.1126/science.1229803

TECHNICAL COMMENTS

Comment and Response on "Climactic Niche Shifts Are Rare Among Terrestrial Plant Invaders"
Comment: B. L. Webber et al.
<http://dx.doi.org/10.1126/science.1225980>
Response: A. Guisan et al.
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Vipers Go Viral
Venomous snakes may hold clues to a deadly virus's recurrence.
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Cows of the Cretaceous
Duck-billed dinosaurs out-chomped the competition.
http://scim.ag/Dinosaurs_Competition

Two Black Holes Inhabit Same Star Cluster
A surprise discovery defies theory, and the same stellar nest may have even more black holes.
<http://scim.ag/Black-Holes>

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9 October issue: <http://scim.ag/ss100912>

RESEARCH ARTICLE: Interferon-Induced SCYL2 Limits Release of HIV-1 by Triggering PP2A-Mediated Dephosphorylation of the Viral Protein Vpu
K. Miyakawa et al.
HIV-infected cells dephosphorylate and inactivate a viral factor to restrict viral spreading.

RESEARCH ARTICLE: Specificity of Linear Motifs That Bind to a Common Mitogen-Activated Protein Kinase Docking Groove
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Three related kinases use structural features to discriminate between potential binding partners.

PRESENTATION: Posttraumatic Stress Disorder in Children and Adolescents—Neuroendocrine Perspectives
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PRESENTATION: Fetal Programming of Brain Development—Intrauterine Stress and Susceptibility to Psychopathology
C. Buss et al.
Intrauterine stress and maternal obesity are associated with neurodevelopmental disorders.

PODCAST
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The neurotrophic factor BDNF has opposite effects on cocaine- and morphine-induced neuroplasticity.

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Free Career Resources for Scientists
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Biomedical Consulting Agreements: The Good, the Bad, and the Ugly
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A leading attorney and a serial entrepreneur tell how to avoid potholes in agreements with industry.

Perspective: Embrace Flexible Work Arrangements
N. Pettorelli et al.
To achieve gender equality in science, shift men's perceptions of what is professionally acceptable.

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Integrating Medicine and Science
10 October issue: <http://scim.ag/stm101012>

RESEARCH ARTICLE: Human Neural Stem Cells Induce Functional Myelination in Mice with Severe Dysmyelination
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Human neural stem cell transplants restore myelination in mice with a hypomyelination disorder.

RESEARCH ARTICLE: Neural Stem Cell Engraftment and Myelination in the Human Brain
N. Gupta et al.
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RESEARCH ARTICLE: Immunotherapy Against HPV16/18 Generates Potent T_H1 and Cytotoxic Cellular Immune Responses
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CD8⁺ T cells with cytolytic activity are induced after therapeutic HPV vaccination in humans.

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S. E. Hyman
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PERSPECTIVE: Next-Generation Treatments for Mental Disorders
T. R. Insel
New approaches are needed for developing next-generation treatments for mental disorders.

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ADVANCING SCIENCE. SERVING SOCIETY



Vesta to the Core

Vesta is one of the largest bodies in the main asteroid belt. Unlike most other asteroids, which are fragments of once larger bodies, Vesta is thought to have survived as a protoplanet since its formation at the beginning of the solar system (see the Perspective by Binzel, published online 20 September). Based on data obtained with the Gamma Ray and Neutron Detector aboard the Dawn spacecraft, **Prettyman *et al.*** (p. 242, published online 20 September) show that Vesta's reputed volatile-poor regolith contains substantial amounts of hydrogen delivered by carbonaceous chondrite impactors. Observations of pitted terrain on Vesta obtained by Dawn's Framing Camera and analyzed by **Denevi *et al.*** (p. 246, published online 20 September), provide evidence for degassing of volatiles and hence the presence of hydrated materials. Finally, paleomagnetic studies by **Fu *et al.*** (p. 238) on a meteorite originating from Vesta suggest that magnetic fields existed on the surface of the asteroid 3.7 billion years ago, supporting the past existence of a magnetic core dynamo.

Distinguishing Right from Left

In most vertebrates during embryonic development, rotational movement of the cilia within a structure in the embryo, known as the node, generates unidirectional flow required for future left-right asymmetry of the internal organs. The flow may transport a determinant molecule or provide mechanical force. However, it is not clear how the flow is sensed.

Yoshida *et al.* (p. 226, published online 13 September; see the Perspective by **Norris and Grimes**) show that nodal flow in mouse embryos is sensed by the cilia of perinodal cells located at the edge of the node, in a manner dependent on Pkd2, a Ca^{2+} -permeable cation channel that has been implicated in polycystic kidney disease in humans.

Bend to Straighten

At low temperatures, the behavior of disordered solids, such as glasses, deviates from that of ordered crystals. The deviations may stem from the ability of some atomic entities to tunnel between two sites of almost identical energy, forming two low-energy states; such two-level systems (TLSs) are also thought to be a major contributor to the decoherence of superconducting qubits. **Grabovskij *et al.*** (p. 232) used mechanical strain to control the splitting between the energy levels of TLSs formed in the disordered barrier of the Josephson junction in a superconducting qubit. For some of the detected TLSs, the splitting exhibited the predicted minimum as a function of strain, verifying the TLS model of disordered solids.

Rise and Subside

Earth's surface tends to deform as magmatic fluids rise toward the surface from below, usually manifesting itself in uplift of continental crust. **Fialko and Pearce** (p. 250; see the Perspective by **Brooks**), however, show that subsidence, or widespread sinking, accompanies uplift when magma rises. Satellite measurements reveal that the massive Altipano-Puna magma body in the central Andes balloons upward, causing subsidence around the region of uplift, resembling a sombrero. Melting of surrounding rocks as the magma rises likely withdraws material and causes the subsidence.

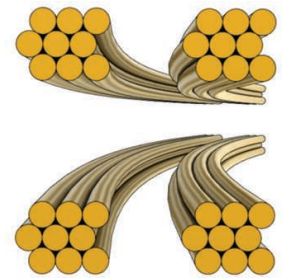
Embryonic Cell Sorting and Movement

Differential cell adhesion has long been thought to drive cell sorting. **Maître *et al.*** (p. 253, published online 23 August) show that cell sorting in zebrafish gastrulation is triggered by differences in the ability of cells to modulate cortex tension at cell-cell contacts, thereby controlling contact expansion. Cell adhesion functions in this process by mechanically coupling the cortices of adhering cells at their contacts, allowing cortex tension to control contact expansion. In zebrafish epiboly,

the enveloping cell layer (EVL)—a surface epithelium formed at the animal pole of the gastrula—gradually spreads over the entire yolk cell to engulf it at the end of gastrulation. **Behrndt *et al.*** (p. 257) show that an actomyosin ring connected to the epithelial margin triggers EVL spreading both by contracting around its circumference and by generating a pulling force through resistance against retrograde actomyosin flow.

Relatively Cold

Temperature is essentially a measure of relative atomic or molecular motion. Low temperature does not necessarily imply a sample at absolute rest—what is important is for every member of the sample to be moving (or not moving) at the same velocity.



Techniques for studying reactions under extreme cooling have nonetheless tended to focus on slowing down molecules. **Henson *et al.*** (p. 234) now demonstrate an alternative approach in which two beams of distinct gas-phase reagents are merged so as to continue forward with very little spread in their velocity. The interactions thus occur at millikelvin temperatures, revealing signatures of nonclassical dynamics such as oscillatory ionization probabilities with small shifts in energy.

The Right Choice?

So-called irrational decisions made by humans are popular fodder for “believe it or not” stories. But what's actually happening when we make choices that do not seem to be justifiable on purely economic or logical grounds? Presumably, we are not simply making errors; instead, our choices may reflect an internal bias that we are not aware of. **Wimmer and Shohamy** (p. 270) show how the hippocampus can instill an unconscious bias in valuations, whereby an object that is not highly valued on its own, increases in value when it becomes implicitly associated with a truly high-value object. As a consequence, we then end up preferring the associated object over a neutral object of equal objective value while not really knowing why.

Continued on page 170

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This Week in *Science*

Continued from page 169

Feeling the Light

There has been a link missing in our understanding of the biochemical signaling mechanism initiated when photons are absorbed by rhodopsin in the photoreceptor cells of *Drosophila* eyes. Photoisomerization of rhodopsin activates a heterotrimeric guanine nucleotide-binding protein which leads to activation of phospholipase C. But how phospholipase C activates the transient receptor potential (TRP) or the transient receptor potential-like (TRPL) ion channels that produce the rest of the cellular response has been unclear. **Hardie and Franze** (p. 260; see the Perspective by **Liman**) propose that there is a physical mechanism at work. Atomic force microscopy revealed light-induced contractions of photoreceptor cells. Consistent with a physical coupling mechanism, manipulations of the membrane altered responses of the photoreceptor cells to light. Thus, physical changes in the membrane appear to couple phospholipase C activity to the opening of mechanosensitive TRP and TRPL channels.

Cheat Control

In quorum-sensing induction, a *Pseudomonas aeruginosa* population growing on a single carbon source, such as casein, will reach a density where the levels of signaling molecules they collectively secrete triggers the cells to synthesize and secrete proteases to digest the casein. However, it is metabolically costly to secrete proteases, and the system is prone to mutant “cheats.” These cheats do not respond to quorum sensing and do not go to the cost of synthesizing protease, but they do profit from the breakdown products that allow all the cells—cheats and cooperators alike—to grow. **Dandekar et al.** (p. 264) found that quorum signaling-insensitive *P. aeruginosa* cheats could not synthesize nucleotide hydrolase and were thus unable to grow if casein was replaced by adenosine. This allowed cooperators to outgrow the cheats, leading to a stable equilibrium between cheats and cooperators. This principle of regulation may be applicable to other bacterial quorum-sensing systems and might be exploited in the development of drugs that disrupt bacterial cooperation.

Ancient Genomics

The Denisovans were archaic humans closely related to Neandertals, whose populations overlapped with the ancestors of modern-day humans. Using a single-stranded library preparation method, **Meyer et al.** (p. 222, published online 30 August) provide a detailed analysis of a high-quality Denisovan genome. The genomic sequence provides evidence for very low rates of heterozygosity in the Denisova, probably not because of recent inbreeding, but instead because of a small population size. The genome sequence also illuminates the relationships between humans and archaics, including Neandertals, and establishes a catalog of genetic changes within the human lineage.



Mobile Phone “Hot Spots”

An obstacle to developing effective national malaria control programs is a lack of understanding of human movements, which are an important component of disease transmission. As mobile phones have become increasingly ubiquitous, it is now possible to collect individual-level, longitudinal data on human movements on a massive scale. **Wesolowski et al.** (p. 267) analyzed mobile phone call data records representing the travel patterns of 15 million mobile phone owners in Kenya over the course of a year. This was combined with a detailed malaria risk map, to estimate malaria parasite movements across the country that could be caused by human movement. This information enabled detailed analysis of parasite sources and sinks between hundreds of local settlements. Estimates were compared with hospital data from Nairobi to show that local pockets of transmission likely occur around the periphery of Nairobi, accounting for locally acquired cases, contrary to the accepted idea that there is no transmission in the capital.



James S. Langer is research professor of physics at the University of California, Santa Barbara. He has served as director of UCSB's Kavli Institute for Theoretical Physics, president of the American Physical Society, and vice president of the U.S. National Academy of Sciences. He is the inaugural editor of the *Annual Review of Condensed Matter Physics*. E-mail: langer@physics.ucsb.edu.

Enabling Scientific Innovation

THESE SHOULD BE WONDERFUL TIMES FOR PHYSICISTS. ADVANCES IN EXPERIMENTAL AND computational capabilities have opened windows to research areas that previously had seemed out of our reach. We now can see in exquisite detail what atoms are doing inside complex materials, and we have the tools to test predictive theories of what's happening. We have been able to do this in ways that are interesting not just to physicists but also to engineers, biologists, and many others. Examples abound in this issue of *Science*, which features a special section that focuses on physics in developmental biology (beginning on p. 209). Unfortunately, our ability to take advantage of these opportunities has been eroded in an environment where innovation and interdisciplinary research are systematically discouraged. Present funding levels in the United States cannot support a scientific community large enough to sustain a full range of world-leading research programs and to educate the new generations of scientists and engineers that the country needs. That problem cannot be solved quickly in today's political climate, but government funding agencies ought not to operate in ways that make it worse.

In my area of condensed-matter and materials physics, the U.S. National Science Foundation (NSF) can fund only about 10% of the individual-investigator proposals it receives. [The Department of Energy (DOE) has similar difficulties.] Each proposal is sent to a group of peer reviewers, who rank it on a scale ranging from "excellent" to "poor." NSF then funds only those proposals that receive the uniformly highest reviews. One less-than-"excellent" review, no matter how misguided, is usually enough to doom a proposal. Any proposal that is truly innovative, interdisciplinary, or otherwise unusual is almost certain to be sent to at least one reviewer who will be less than enthusiastic about it. Sensible investigators know not to submit such proposals; as a result, some of the most urgent research areas are disappearing. Consider, for example, the behavior of materials that are driven away from thermodynamic equilibrium. We need to learn how to predict irreversible responses to strong forces for structures ranging from jet engines to biological membranes. These are difficult, deeply fundamental, and intrinsically interdisciplinary research problems. But far too little attention is paid to them these days.

The U.S. system for funding research was designed 60 years ago to function well in times of growth. It is failing now, not because peer review is failing, but because the system as a whole is contracting. Much of today's proposal pressure is caused by underfunded investigators who must compete for multiple grants to survive professionally. They do this under stress from promotion committees and with growing uncertainty about peer review. Such scientists have little time or incentive to be innovative.

What is to be done? The funding agencies, especially NSF and the DOE, must admit that it is not humanly possible to predict, with high accuracy, which research projects ultimately will have the most impact. Peer review, at best, can identify fundable proposals. When there are too many of these, as at present, the agencies must find other ways to decide which to support. That will be hard.

The United States must not abandon the spirit of peer review by relegating these hard decisions to a bureaucracy. The easy alternative would be to choose among the fundable proposals by a lottery. Strange as it seems, that could well do less damage than the present system; but it would be a mindless process, unworthy of U.S. science. Perhaps the funding agencies and the scientific community together can devise a mechanism for assessing the urgency of the problem, setting priorities, and finding compromises. An independent agency such as the U.S. National Academies could coordinate this process. At the very least, it will be critical to find modes of operation that do not discourage the most imaginative investigators just because their proposals are too innovative.

— James S. Langer





ECOLOGY

Don't Touch My Cache

For plants, seed dispersal is critical for survival. It can provide an escape from competition, disease, and predators. One way seeds are dispersed is by seed-caching predators—their few forgotten seeds may eventually germinate and grow into new plants. One concern, however, is that although these animals move seeds away from competition with their parent plant, they may place them in competition with other conspecifics nearby. Hirsch *et al.* addressed this by following hundreds of radio-tagged black palm seeds for over a year on Barro Colorado Island. They found, instead, that the tree's main disperser, the Central American agouti, distributes its seeds to areas with low conspecific density. The distance from the parental tree and other conspecifics increased the more the seed was moved by agoutis, up to 18 times in some cases. The agoutis may be selecting areas of low black palm density for their caches, and the subsequent moving of their seeds, in order to avoid pilfering by other agoutis. Thus, the efforts of the agoutis to protect their caches result in better germination conditions for the trees and are probably worth the cost of the seeds that aren't forgotten. — SNV

Ecol. Lett. **15**, 10.1111/ele.12000 (2012).

GENETICS

How to Part Ways

Proteins perform critical functions in the cell, serving as structural units, enzymes, and transcription factors, among other roles. Protein activity can be static, as for some structural proteins, or dynamic, as in the case of transcription factor binding to gene promoters. In many cases, once transcription begins, transcription factor binding is no longer needed and in fact needs to be terminated so that other regulatory factors can act at that site. How this occurs, however, is not well understood. Zelin *et al.* now provide one such example. They show that the molecular chaperone p23 functions to disassemble protein-DNA complexes that include heat shock factor 1 or the DNA replication factor CDC6. Subsequently, covalent modification via acetylation of lysine residues by GCN1 can extend this dissociated state. Such dynamic interplay among a multitude of factors provides insight into how complex DNA regulation is mediated. — BAP

Mol. Cell **47**, 10.1016/j.molcel.2012.08.026 (2012).

EDUCATION

How to Train a Leader

If we are to increase the number of women in science, technology, engineering, math, and medicine (STEMM), it is imperative to increase the number of women in STEMM leadership roles. Isacc *et al.* describe the impact of a 16-week course that focused on increasing women's lead-

ership self-efficacy with the aim of making the students "bias literate." In the course, students discussed how gender stereotypes influence behavior and were presented with evidence-based strategies designed to counteract their impact. Pre- and post-course surveys were evaluated from the first three cohorts along with follow-up queries of the first two cohorts, with quantitative analyses of differences between scores for all measures being significant. Increases in scores for leadership self-efficacy and personal mastery, coupled with a decrease in perceived constraints, suggested that the course prepared participants to engage in leadership. Analysis of journal entries showed that participants recognized their own implicit gender bias while citing research discussed in class. Also prevalent in journal entries were narratives indicating a new view on leadership—specifically, the realization that the participants themselves were capable of being successful leaders. — MM

CBE Life Sci. Educ. **11**, 307 (2012).



GEOPHYSICS

Hitching a Ride into the Mantle

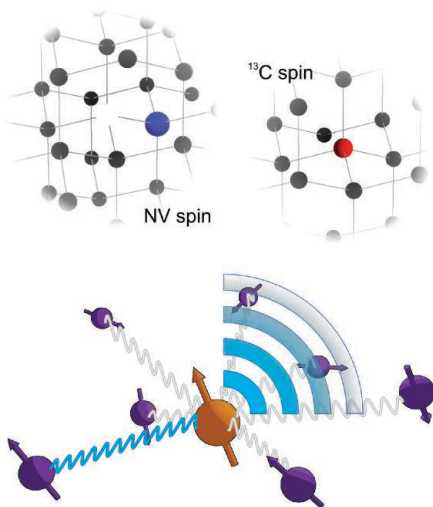
The movement of Earth's crust relies on the underlying mantle to behave viscously and flow as a response to convective forces. As a consequence, the upper mantle just below the oceanic crust may get pulled along with the crust as it is driven to depth at subduction zones. Alternatively, the mantle may decouple from the subducting crust as a result of mantle flow in another direction. One way to track the flow of the mantle is through monitoring how seismic waves respond to the orientation of certain mineral grains, which preferentially align according to flow directions and induce anisotropic splitting of seismic waves. Song and Kawakatsu examined this seismic anisotropy below the global oceanic asthenosphere—the viscous and ductile upper layer of the mantle—below subduction zones as a function of subducting slab dip and incident angles. They found that the times and direction of seismic shear waves split into fast and slow components in a way that is consistent with about 100 km of the asthenosphere flowing as though it is coupled to the subducting slab even with buoyancy forces resisting its downward motion. Appreciable subduction of oceanic asthenosphere over long time scales would require the revision of mantle mixing models. — NW

Geophys. Res. Lett. **39**, L17301 (2012).

PHYSICS

Exercising Spin Control

Electron spin resonance is a powerful tool that can provide detailed information on the chemical and electronic makeup of a material. Compared to the typical measurements of bulk samples that are ensemble averages of many electron spins, the nitrogen vacancy defect, or NV center, in diamond presents the spin of a single electron. Such pinpoint spin centers can be used as a local probe of the tiny magnetic fields produced by nearby nuclear spins of the diamond lattice surrounding it and are also



being explored for applications in quantum optics and quantum information science. Because the local environment of each nuclear spin is slightly different, each has its own electronic signature. Kolkowitz *et al.* and Taminiau *et al.* exploit that to demonstrate that they can address and coherently control each of the surrounding nuclear spins through their specific coupling with the NV center. The ability to sense and control the electronic properties of these NV centers and surrounding nuclear spins will have wide application, from ultrasensitive magnetometers to quantum information processing in which information can be encoded in the spin of the surrounding nuclear spins. — ISO

Phys. Rev. Lett. **109**, 137601; 137602 (2012).

MICROBIOLOGY

Hit 'Em Quick, Hit 'Em Strong

Clinicians are very aware of the increasing failure rate of currently available antibiotics to treat persistent infections. In fact, we know little about how populations of bacteria respond to antibiotics, but we do know that the efficacy of

antibiotics in several classes depends on the initial density of the bacteria at infected sites, regardless of whether resistant mutants arise or whether protective mechanisms are triggered in the pathogens. It makes sense that the presence of a greater number of bacteria capable of breaking down a fixed dose of antibiotic will allow a greater proportion to escape the effects of the drug. But what happens if the bacteria can't disable the antibiotic? Tan *et al.* took kanamycin, an antibiotic that targets the bacterial ribosome, and discovered that this drug indirectly induces a heat shock response to the mistranslated proteins, which degrades the ribosome. The denser the bacterial population, the more the antibiotic is titrated and the greater the chances that more bacteria will escape after a period of recovery, or lag, to regrow. It's not so simple though, because clinicians often administer pulses of antibiotic to avoid toxicity effects on patients and the emergence of resistance. But it is also important that they get the dose and treatment times optimized around the lag times, otherwise bacteria may escape again. — CA

Mol. Syst. Biol. **8**, 10.1038/msb.2012.49 (2012).

ECONOMICS

Hard Data Help, a Little Bit

Performance appraisals are generally not high on anyone's list of likes and are often points of friction between employers and employees. One educational program in the United States, Race to the Top, has encouraged the use of student outcomes—in the form of scores on standardized tests—in the evaluations of teachers. Rockoff *et al.* have analyzed the results of a randomized trial carried out in the New York City public schools, where the difference between treatment and control groups of school principals was the provision of value-added measures of individual teacher performance to the former. They found that the hard data did correlate with the principals' prior subjective evaluations of their teachers; moreover, the agreement was greater when the hard data were more precise or when the principals had been observing the teachers for more years—as one would hope and expect. Also, the principals weighted the hard data more strongly in cases where their subjective evaluations were less reliable, and teachers who received lower-value-added reports were more likely to be let go. On the critical issue of student performance, however, the outcomes were modest: Math achievement went up a little bit, and English achievement stayed the same. — GJC

Am. Econ. Rev., in press (2012);
www.nber.org/papers/w16240.

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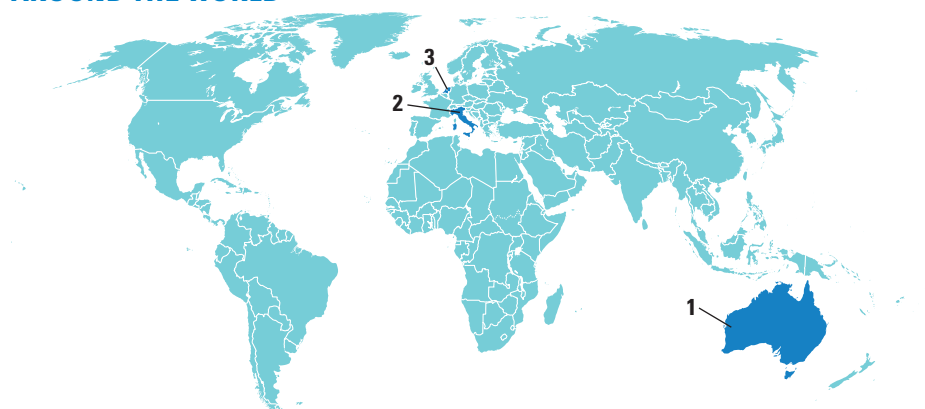
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AROUND THE WORLD



Boolardy Station, Australia 1

New Radio Telescope Takes Aim

“Exotic objects that push the boundaries of our knowledge of the physical laws,” are the target of the Australian Square Kilometre Array Pathfinder (ASKAP), says astrophysicist Brian Boyle, of Australia’s Commonwealth Scientific and Industrial Research Organisation.

The \$400 million ASKAP, completed last week at the Murchison Radio-astronomy Observatory in Western Australia, comprises 36 12-meter-diameter antennas. With its wide view of the sky and



high-speed data acquisition, Boyle says, “in one day ASKAP will gather more information than is in all the radio astronomy archives around the world today.” During its first 5 years of operation, the observatory will take a census of galaxies within 2 billion light-years of Earth, study cosmic magnetic fields, and search for black holes.

Boyle says scientific observations will start by the end of the year with the first results likely within 12 months. And then, beginning in 2016, an additional 60 dishes

will turn ASKAP into part of the world’s largest radio telescope—the Square Kilometre Array (*Science*, 1 June, p. 1085).

Parma, Italy 2

European Food Agency Pans Contested GM Study

An expert panel convened by the European Food Safety Authority (EFSA) has dismissed as “inconclusive” a controversial study claiming to find that rats fed genetically modified maize developed tumors at a higher rate than control animals (*Science*, 28 September, p. 1588). The study “is of insufficient scientific quality to be considered as valid for risk assessment,” the group concluded in a 4 October report. But EFSA invited the study’s lead author, Gilles-Eric Seralini of the University of Caen in France, to provide the agency with more information by 12 October. “We will put our raw data on a public Web site,” Seralini told *Science*. He says he’d like to see EFSA do the same with the data they used to approve the GM variety. Meanwhile, a separate review by Germany’s Federal Institute for Risk Assessment also found the study wanting, and French authorities are expected to offer their views on 20 October.

Tilburg, the Netherlands 3

Stapel Under Investigation By Dutch Prosecutors

Disgraced Dutch psychologist Diederik Stapel is under investigation by the Dutch Public Prosecution Service and the Fiscal Information and Investigation Service, according to media reports in the Netherlands.

NRC Handelsblad reported last week that investigators are trying to establish whether Stapel defrauded the gov-

NOTED

>Schools in England are failing girls who want to study physics, says a new report from the United Kingdom’s Institute of Physics. Almost half (49%) of mixed state-funded high schools did not have a single girl continuing physics beyond age 16, according to 2011 exam results. **Girls are two-and-a-half times more likely to continue with physics if they attend a single-sex school.**

ernment by collecting grant money for research he did not carry out and made false statements in his accounts of how the money was spent.

Stapel resigned from Tilburg University in 2011 after an investigation revealed that he made up the results in many of his eye-catching social psychology studies (*Science*, 4 November 2011, p. 579).



Stapel

So far, 25 of his papers have been retracted; a commission is still investigating others. *NRC Handelsblad* reports that Stapel received €2.2 million in grants from the Netherlands

Organisation for Scientific Research, and university funds may be part of the inquiry as well. Officials will decide whether to prosecute Stapel once the investigation is complete, which could take months.

<http://scim.ag/stapelfraud>

NEWSMAKERS

Ten Scientists Receive Genius Grants

The John D. and Catherine T. MacArthur Foundation last week announced the winners of its annual MacArthur Fellowships, otherwise known as Genius Grants, including 10 scientists. The \$500,000 awards are unusual in that there is no public nomination process. A private committee reviews anonymous nominations, and the foundation’s president and board of directors select the final winners. The awardees don’t know they are in the running until they receive a congratulatory phone call from the foundation.

Awardee **Terry Plank**, a geochemist



Random Sample

Wikipedia Edit-a-Thon
For Female Scientists

Search Wikipedia for “computer programming” and you will discover Ada Lovelace, a 19th century English mathematician whose notes expanding on Charles Babbage’s description of the first general-purpose mechanical computer have earned her widespread recognition as the world’s first computer programmer. Lovelace remains one of few historical female scientists to win such distinction for her work—a problem that the Royal Society in London hopes to remedy on Wikipedia in an “edit-a-thon” event on 19 October. Fifteen female editors will create or expand Wikipedia articles on illustrious yet unsung female scientists using archives from the Royal Society’s library.

at Columbia University who studies the intersection of hydrology and volcanology, received the “famous phone call out of the sky” while driving to pick her son up from school. “I’m still in shock that someone said, ‘Here’s half a million dollars, no strings attached,’” she says. “It’s better than the lottery.” Plank plans to spend the money on some riskier science experiments that might otherwise never receive funding.

See the full list of winners at www.macfound.org/fellows/class/2012/.

When Fanged Dwarf Dinosaurs
Roamed the Earth

Long before *Triceratops* stomped the earth, a family of dinosaurs called heterodontosaurs scamped about the supercontinent Pangaea armed with vampirelike fangs. Some paleontologists argued that the teeth showed the creatures ate insects or small animals as well as plants. Others claimed the fangs were mostly for show, used to spar with rivals or scare predators. Now, a 60-centimeter-tall heterodontosaur called *Pegomastax africanus*, described online last week in *ZooKeys*, may settle the debate. Microscopic analysis of tooth wear, and the reconstruction of a close cousin, suggest *Pegomastax* used its choppers to nip and spar, and not for wholesale meat-eating.



FINDINGS

The Cost of Biodiversity

Conservation funding worldwide must increase by an order of magnitude to meet international targets for safeguarding biodiversity by 2020. A paper published online in *Science* this week unveils this first-ever global biodiversity price tag: \$78 billion dollars a year.

To calculate the cost, the researchers leaned heavily on data about birds, the best known taxa. On average, threatened bird species receive only 12% of the funding needed to lower their risk of extinction; and just 28% of the most important habitat is completely protected. Adding up full funding for those programs and for similar programs for other organisms (using comparative data about conservation costs) yielded the final tally.

That cost of conservation is vastly outweighed by the potential ecosystem benefits to humans, says Stuart Butchart, global research coordinator for BirdLife International in Cambridge, U.K.

The 2020 targets were set by the Convention on Biological Diversity in 2010 (*Science*, 10 September 2010, p. 1272). Convention delegates are meeting in Hyderabad, India, until 19 October to figure out how to achieve these targets.

Vipers Go Viral

Every spring, the Eastern equine encephalitis virus (EEEV) reemerges in the eastern United States, where it can cause devastating disease in horses and humans. Scientists have wondered how the virus, which is transmitted by mosquitoes, survives the winter. The answer is snakes, a new study suggests. A team led by microbiologist Thomas Unnasch of the University of South Florida in Tampa found signs of the virus in two venomous species—the cottonmouth and the copperhead—collected in Alabama’s



Tuskegee National Forest. And experiments with less dangerous garter snakes showed that EEEV could hang out while they hibernated, researchers reported last week in *The American Journal of Tropical Medicine and Hygiene*. Now, says virologist Laura Kramer of the New York State Department of Health’s Wadsworth Center in Albany, they have to prove “the last little piece of this puzzle”—that snakes are infecting mosquitoes in the wild. <http://scim.ag/vipersviral>

NOBEL PRIZE IN PHYSIOLOGY OR MEDICINE

Reprogrammed Cells Earn Biologists Top Honor

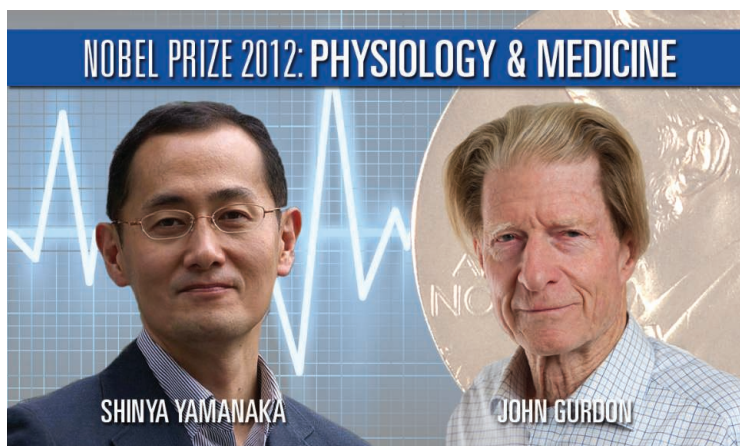
Life is a one-way trip. Plants and animals start as embryos and progress through irreversible developmental stages to eventual death. It was natural for early biologists to assume that this inexorable law of life applied to all the cells that make up these organisms, and that a cell's differentiation and maturation could not be reversed. But a pair of researchers a generation apart thoroughly upended that thinking. The first showed that a nucleus from a mature, specialized cell could be made to restart development as if it were young; the second, nearly 4 decades later, found that adding a few genes to mature cells in a laboratory dish could prompt them to behave like cells in early embryos.

Demonstrating that this reprogramming could turn back the clock at the cellular level has netted the pair—John Gurdon, a developmental biologist at the University of Cambridge in the United Kingdom, and Shinya Yamanaka, a stem cell researcher at Kyoto University in Japan and the University of California, San Francisco—this year's Nobel Prize in physiology or medicine. The pair's work “revolutionised our understanding of how cells and organisms develop,” the Nobel committee wrote in its award announcement. The two discoveries “are like bookends” to the field of cellular reprogramming, says Alan Colman, a stem cell researcher at the Institute of Medical Biology in Singapore. “The [Nobel] committee got it exactly right.”

The ability to reprogram adult cells has made it possible for researchers to study diseases in new ways and raises the possibility of someday growing replacement tissues or even organs in the lab. Yamanaka's work, published just 6 years ago, “is completely transformative,” says Austin Smith, a developmental biologist at the University of Cambridge. Although the Nobel Committee often waits decades before awarding a prize, in Yamanaka's case, Smith says,

“the impact has been so big and so rapid, it's entirely appropriate.”

Gurdon, now 79, waited a bit longer for the committee's recognition. In the early 1960s, working at the University of Oxford, he showed that under the right conditions, a mature cell nucleus could become developmentally young again. He replaced the nucleus of a frog egg with a nucleus taken from a cell in a tadpole's intestine. Most of the time, the egg did not develop successfully. In a few cases, the egg cell was able to reprogram the DNA in the tadpole nucleus



and the egg cell produced a normal frog—the first animals cloned from mature cells. Gurdon described the key experiments in 1962 in the *Journal of Embryology and Experimental Morphology*. Researchers once thought that cells might shed some DNA as they matured, Colman notes, but Gurdon's work disproved that.

Other researchers built on Gurdon's findings, most famously the team that cloned Dolly the sheep using a similar feat of nuclear transfer. That breakthrough demonstrated that mammalian cells could undergo the same transformation from mature to immature. The technique was extremely inefficient, however, resulting in many more failures than successes. And no one has been able to get the method to work in human cells.

A fundamental question dogged the field: Could researchers reconstitute in a lab dish

an egg cell's ability to turn back the cellular clock? “What we all believed ... was that it could be done but must be a very, very complicated process,” Colman says. “Then Shinya comes along and showed only four factors could do it.”

Yamanaka, now 50, and his colleagues started by adding extra copies of more than 20 embryonic and developmental genes to mouse skin cells growing in lab dishes. A few of the treated cells began to act like embryonic stem (ES) cells. (ES cells, taken from early embryos, are pluripotent, which means they can become any of the body's tissue types.) Through a process of elimination, the researchers found that adding just four genes would do the trick, a finding they reported in *Cell* in 2006.

A year later, Yamanaka and other teams showed that a similar technique could work on human cells. That has allowed scientists to establish stable, growing populations of cells from patients with diseases such as amyotrophic lateral sclerosis (ALS). Researchers can study such cells, known as induced pluripotent stem (iPS) cells, for disease insights. In the case of ALS, for example, they can prompt them to become muscle and nerve cells that mimic the problems seen in people with the condition. Scientists hope that such “disease in a dish” models can be used to discover potential treatments.

Yamanaka's work was also welcomed because it offers researchers a potential alternative to human ES cells, which have been ethically and politically controversial given their source. In 2008, Yamanaka told *Science* that his work was in part inspired by the view of an embryo through a microscope at a friend's fertility clinic. It reminded him of his two daughters, he said (*Science*, 1 February 2008, p. 562).

Researchers have found several variations on Yamanaka's original recipe for making iPS cells, which involved inserting extra copies of genes that are involved in cancer; the resulting cells were prone to forming tumors. It's now possible to reprogram adult cells to a stem cell state without making permanent genetic changes in them. Several groups are working on ways to reprogram mature cells—say, skin cells—directly into another mature cell type such as cardiac

CREDITS: (LEFT) CREATIVE COMMONS; (RIGHT) WELLCOME LIBRARY, LONDON; (BACKGROUND) ISTOCKPHOTO.COM; NOBEL FOUNDATION

Downloaded from www.sciencemag.org on October 12, 2012

cells, skipping the pluripotent state.

Scientists are still working to understand how cells reprogrammed by the addition of genes differ from the pluripotent cells in embryos, and how those differences might affect the ways the cells can be used. And no one yet knows exactly how the reprogramming factors work their cellular magic.

Even as the basic research continues, “the application of iPS cells in studying human gene function, in disease model-

ing, and in drug development and toxicology is well under way and continues to accelerate,” says Martin Pera, a stem cell biologist at the University of Melbourne in Australia. One clinical trial using iPS cells to treat macular degeneration is slated to start in Japan next year.

Gurdon released a statement highlighting how the two scientists’ research had begun to move from basic science into medicine: “It is particularly pleasing to see how

purely basic research, originally aimed at testing the genetic identity of different cell types in the body, has turned out to have clear human health prospects.”

In a Japanese TV interview, Yamanaka also addressed the field’s medical future, saying he felt “a great responsibility” to develop new therapies and drugs. “I feel we have to continue research to make a contribution to society as early as possible.”

—GRETCHEN VOGEL AND DENNIS NORMILE

NOBEL PRIZE IN PHYSICS

Manipulators of the Quantum Realm Lauded

The past couple of decades have seen a sea change in quantum physics. Previously, scientists relied on the strange rules of quantum theory mainly to explain the odd natural behavior of masses of atoms and other quantum particles such as photons. Increasingly, however, physicists are exploiting those rules to create delicate quantum states of individual particles and to do novel things with them. This year’s Nobel Prize in physics honors two experimenters who have helped blaze that trail.

Serge Haroche, 68, of the Collège de France and the École Normale Supérieure, both in Paris, pioneered the sculpting of a quantum state of light and the control of its interactions with a single atom. David Wineland, who is also 68, of the National Institute of Standards and Technology in Boulder, Colorado, developed techniques for controlling individual charged atoms, or ions, and showed how they could be used to perform computations. “Forty years ago it wasn’t even clear that quantum mechanics would apply to single particles because it’s a statistical theory,” says Ignacio Cirac, a theorist at the Max Planck Institute of Quantum Optics in Garching, Germany. “They opened the door to studying individual quantum systems.”

Haroche helped pioneer the field of “cavity quantum electrodynamics,” in which researchers precisely tune the quantum state of light that’s trapped between two mirrors. That state can be probed by sending a single atom through the light.

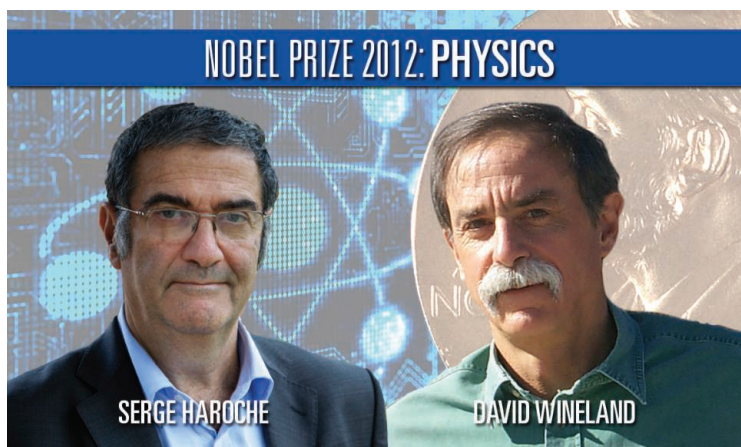
Among other things, Haroche and colleagues demonstrated for the first time perhaps the weirdest of quantum states: the Schrödinger’s cat state. Quantum theory allows a physical system to be in two mutually exclusive states at once, and in 1935 Austrian theorist Erwin Schrödinger dreamed up a scheme to force a cat hidden in a box to be both alive and dead at the same time. (As soon as the box is opened, the quantum state “collapses” so that the cat is found either dead or alive.) In 1996, Haroche and colleagues created a similar two-ways-at-once state of light. “It was one of the most exciting experiments

lems that would overwhelm a classical computer. “David has really come up with all the first big advances in this field,” says Winfried Hensinger of the University of Sussex in the United Kingdom.

Ions spin like little tops and, using lasers, researchers can control an ion’s spin state, even putting it into a delicate two-ways-at-once quantum state. Wineland and colleagues transferred the information in such a spin state to the jiggling of the ion in the electric field that holds it. They even made neighboring ions interact and communicate through such jiggling. Using those techniques, the researchers were able to use a single ion to perform an elementary operation in logic, as they reported in 1995. A full-fledged quantum computer is still years away, but physicists have used as many as 15 ions together to do computations. That’s about a quarter of what’s needed for a quantum computer to surpass a classical computer for some limited set of problems, Hensinger says.

As with many Nobel prizes, observers say that other researchers might have merited the award, too. In the field of ion trapping Christopher Monroe of the University of Maryland, College Park, and Rainer Blatt of the University of Innsbruck in Austria have also made seminal contributions, Hensinger says. Varcoe says that in cavity quantum electrodynamics, Jeff Kimble of the California Institute of Technology in Pasadena and the late Herbert Walther of the Max Planck Institute of Quantum Optics have also been guiding lights.

—ADRIAN CHO



I’ve ever seen and is actually the reason I’m in this field,” says Benjamin Varcoe, a quantum physicist at the University of Leeds in the United Kingdom.

Wineland and his team developed myriad techniques to trap and control individual ions. Although trapped ions could be used to make much better atomic clocks, the real attraction of Wineland’s work is that it could someday be used to create a quantum computer that would be able to solve certain prob-

lems that would overwhelm a classical computer. “David has really come up with all the first big advances in this field,” says Winfried Hensinger of the University of Sussex in the United Kingdom.

MARINE CONSERVATION

Oh, Baby: Fight Brews Over U.S. Import of Beluga Whales

In the Bay of Sakhalin off Russia's eastern coast, 18 wild beluga whales captured in the Sea of Okhotsk are circling in sea pens, awaiting a U.S. government decision that could send them on a 12,000-kilometer journey. Earlier this year, the Georgia Aquarium in Atlanta made a controversial request to the U.S. National Oceanic and Atmospheric Administration's (NOAA's) Fisheries Service: It wants a permit to load the Russian whales into cargo jets and ship them to the United States for display and breeding in Atlanta and five other cities. The request is the first of its kind since 1988, and it is drawing extensive opposition from animal-rights advocates and some whale research-

Of the 31 beluga whales currently in captivity in the United States, only three were directly captured from the wild for the purpose of display—all from the Canadian Arctic in the late 1980s, according to NOAA records. The rest were transferred to their current homes from other facilities or rescued after becoming stranded, injured, or sick. That pattern reflects public opposition in the United States to capturing wild belugas and a growing recognition among scientists that the whales need more space and social interaction than most marine parks and aquaria can provide, says whale specialist Scott Kraus of the New England Aquarium in Boston.

biology of these important animals," says Brandon Southall, a former NOAA bioacoustics researcher who supports the permit. Now a research associate with the University of California, Santa Cruz, Southall studies how marine mammals respond to potentially disruptive sounds created by devices such as military sonar, and he says work with captives could help identify ways of reducing threats. And "the data show that animals like belugas and bottlenose dolphins do breed in captivity," says the New England Aquarium's Kraus, suggesting that aquaria "could theoretically create a captive colony, given adequate resources."

Still, Kraus is one of many scientists who have doubts about the plan's claimed benefits. For instance, Kraus says that although aquaria can play a role in promoting ocean conservation, large marine mammals "are almost certainly not" necessary to get people interested. And there's no reason to believe that the difficult task of breeding captive belugas will get easier with fresh animals, says Naomi Rose, a biologist with Humane Society International in Gaithersburg, Maryland. She is also skeptical that research on captive whales will benefit their wild relatives, because the biggest threat to belugas is loss of habitat and food due to global warming. "I can't imagine what studies they could design in captivity that would address those concerns," she says.

Such issues, as well as questions about how the belugas were captured and whether they can be transported and kept humanely, are likely to be hotly debated at the NOAA hearing. The agency has already received more than 4000 comments on the permit request and recently extended the public comment period to 29 October because of high interest. A decision isn't expected until January at the earliest.

In the meantime, some marine mammal researchers worry that the fight is a distraction from bigger issues facing marine ecosystems. "Every time somebody does something like this, there's a huge controversy," Kraus says. But there's less public outcry about the numerous marine mammals that die after becoming entangled in fishing gear or the extensive impacts of development and pollution on coastal seas. "These are the things that are killing the oceans," he says. "Eighteen belugas out of the Okhotsk Sea? That's kind of a drop in the bucket."

—EMILY UNDERWOOD



A great white whale. Beluga whales, such as this one on display at the Georgia Aquarium in Atlanta, are a favorite with visitors.

ers. This week, the conflict is expected to get its first formal airing, with NOAA officials scheduled to hold a 12 October hearing at the agency's headquarters in Silver Spring, Maryland.

Growing up to 4.5 meters long, beluga whales are toothy, pearly white mammals that live in the Arctic Ocean. More than 150,000 belugas exist worldwide, researchers estimate; just one population of about 350 in Alaska is considered endangered under U.S. law. The creatures' whistles and chirps, and a playful tendency to spit water at visitors, make them an aquarium favorite. Their curious appearance has also inspired best-selling children's songs, books, and even a boutique.

the application notes. In part, that's because U.S. facilities have struggled to breed the animals successfully; the Atlanta aquarium's first captive-born calf, for instance, died this past May. The aquarium, which currently owns four belugas, says it plans to keep "approximately" six of the 18 animals and loan the rest to other facilities. Once in their new homes, the whales will help promote marine conservation among hundreds of thousands of visitors, the aquarium says, and enable scientists to pursue research that is hard to do in the wild, such as up-close studies of physiology and behavior.

Those are reasonable arguments, say some whale researchers. "This is a unique opportunity to learn more about the phys-



BIOMEDICAL RESEARCH POLICY

NIH Funding Shifts With Disease Lobbying, Study Suggests

The growing power and influence of disease lobbying groups over the past 2 decades have dramatically reshaped the priorities of the U.S. National Institutes of Health (NIH), according to a new analysis. The study, which has drawn some skepticism, finds that the more money a group spent on lobbying, the more NIH boosted its spending on that disease.

The findings “suggest that advocacy has an impressive payoff,” says sociologist Steven Epstein of Northwestern University in Evanston, Illinois, who did not participate in the study. He also describes as “an important shift” the study’s finding that lobbyists have nudged NIH closer to allocating funding by dollars per disease death, a direction he supports.

NIH leaders have long argued that they should base funding decisions mainly on the scientific quality of proposals, rather than by targeting funds to specific diseases. But pressure to target funds grew in the 1980s and 1990s with the rise of patient lobbying groups such as those focused on diabetes and Parkinson’s disease. Lobbyists for breast cancer and AIDS research, in particular, have achieved dramatic increases in federal funding at NIH and, for breast cancer, at the Department of Defense (DOD). University of California (UC), Berkeley, sociology graduate student Rachel Kahn Best wanted to look across diseases to see how these efforts shaped federal spending.

So Best gathered data on budgets and lobbying activities of disease nonprofits, disease funding data from NIH, and U.S. death statistics for 53 diseases. She focused on spending at NIH and DOD from 1989 to 2007 for the diseases, including multiple

sclerosis and major killers such as influenza and heart disease. The dollar total for these 53 diseases at NIH came to more than \$10 billion in 2005, or about 35% of NIH’s then-\$28.5 billion budget. (Because breast cancer and AIDS have such outsize influence, she excluded them from the analysis, and her list includes only a few rare diseases such as cystic fibrosis.)

Advocacy paid off, Best found: For every \$1000 a group spent on lobbying, NIH and DOD spent an average of \$25,000 more on that disease the following year. (The trend was similar when Best excluded DOD funds.)

Although some patient groups may laud these trends, the downside is that diseases with smaller lobbying efforts, such as pancreatic cancer, which leaves few survivors, lagged behind, Best says. “The risk is that we’ll neglect diseases with scientific potential and big public health benefits,” she says. She also found that two common diseases that carry a stigma—lung cancer and liver cancer—didn’t do as well in the new funding environment. Best, now a postdoc at the University of Michigan, Ann Arbor, published the study in the October issue of *American Sociological Review*.

The study also found another way that disease lobbyists influenced NIH’s priorities. One argument these advocates began making in the 1980s—and that lawmakers picked up—was that NIH’s spending per patient death from the major killers they were lobbying for fell far short of spending per death from AIDS. In 1998, the Institute of Medicine also urged NIH to take into account the burden of disease. Best found that, as a result, NIH’s increases in spending per disease over the 19 years increasingly favored

Dollars for dollars. Parkinson’s and other patient groups have nudged up NIH funding for their diseases.

high-mortality diseases, although AIDS still gets far more per death than other diseases. “Once advocates started making mortality claims, diseases that were big killers tended to get larger funding increases,” Best says.

Although the results aren’t surprising, others say, this is the first study to quantify how some diseases have benefited more from advocacy than others. “Most people were aware of the effects, but it was all based on anecdotes,” says sociologist David Hess of Vanderbilt University in Nashville. “It shows that disease-focused nonprofits are doing their work well,” agrees epidemiologist Claiborne Johnston of UC San Francisco, who has also found a strong link between charity revenue and NIH disease funding.

But not everyone buys the analysis. Howard Garrison, deputy executive director for policy at the Federation of American Societies for Experimental Biology, questions whether NIH’s data on disease funding are accurate. Before 2007, the agency often only tallied spending on a specific disease when a lawmaker asked about it. “It’s a politically driven data-collection process,” and that could have skewed the numbers, Garrison says. He also notes that many patient groups don’t push for targeted funding but for increases in NIH’s total budget.

Another question is how the shifts happened. Congress rarely earmarks funds for specific diseases in NIH’s budget, so the reasons why the agency adjusted its spending priorities aren’t clear, Best admits. Factors might include congressional report language urging NIH to pay more attention to a disease and researchers shifting their attention to a new area in response to lobbying, she says. “My guess is that several mechanisms play a role.”

Garrison says he sees little evidence in NIH’s grants data that it is funding more targeted grants: “It doesn’t hold together with what I know about the way the NIH operates.” Still, if the trend Best identifies is real, Garrison agrees it has troubling implications: that by giving in to pressure to fund specific diseases, NIH is likely shortchanging certain areas, including basic research, infrastructure, and support for young scientists. And as NIH’s budget has leveled off in the past few years, Best notes, competition has likely grown even fiercer. “If this [NIH funding] degenerates into a narrow fight for resources, that would be wasteful and ignore scientific opportunity,” Garrison says.

—JOCELYN KAISER

U.S. POLAR SCIENCE

New Arctic Research Vessel Ready to Make a Splash

Polar researchers will soon have their own ride to the icy waters of the Bering Sea and points north. After more than a decade of hitchhiking aboard the *Healy*, a U.S. Coast Guard icebreaker that doubles as a research vessel, marine scientists are celebrating the launch this weekend of the *Sikuliaq*, a \$200 million, nearly 80-meter ship owned by the National Science Foundation (NSF). “It’s been a long time in coming,” says Jacqueline Grebmeier, a biological oceanographer at the University of Maryland’s Chesapeake Biological Laboratory in Solomons.

The *Sikuliaq*, named for an Inupiaq word meaning “young sea ice,” could help usher in a new era in Arctic research. It offers dedicated ship time to researchers studying climate change, nutrient distributions, and migration patterns in the Arctic’s constantly changing marginal ice zones, where open water transitions into pack ice.

Although not technically an icebreaker, the *Sikuliaq* is powerful enough to push its way through meter-thick masses of relatively fragile first-year sea ice at a speed of 2 knots. For researchers, that means the chance to work year-round in the Bering Sea, south of the Bering Strait. In the summer (and possibly spring and fall), the ship can also go into the marginal ice zone of the Chukchi Sea and other regions north of the Bering Strait and Alaska.

“The ship is really designed around the science,” says Daniel Oliver, project manager and director of the Seward Marine Center at the University of Alaska, Fairbanks (UAF), which will operate the vessel. Because Arctic research is inherently multidisciplinary, encompassing diverse issues such as fisheries and ecosystem research and monitoring volcanoes in the Aleutian arc, the ship needed to be adaptable. One key feature is the ship’s 30 meters of open deck space, Oliver says. “There’s a great deal of flexibility for bringing special labs on, carrying a lot of moorings or buoys,” or even to retrieve 20-meter-long cores from the sea floor.

The ship also has multiple mapping systems mounted on a retractable centerboard in the ship’s hull: one for shallow- and medium-depth acoustic mapping and another for mapping sea floor topography

and imaging rock strata beneath the sea floor. To make room for all of this, the ship is twice the length of its predecessor, the *Alpha Helix*, which operated for 40 years before it was sold in 2007.

“The lack of an ice-capable vessel dedicated for the science community has been one of the biggest factors” in pushing plans for the *Sikuliaq* forward, says Terry Whitledge, a marine chemist at UAF and the chair of the vessel’s design committee. By the 1990s, it was clear that the aging

began in 1998, but by 2005, when the design was ready, NSF’s construction account was filled with other projects.

The logjam was broken in 2009, when NSF received a one-time \$400 million appropriation for new facilities as part of the massive 2009 federal stimulus package. Then-NSF Director Arden Bement chose to spend \$148 million of it on the *Sikuliaq*.

Timingwise, “it was absolutely ideal,” says Matt Hawkins of NSF’s ocean sciences division. “Congress asked about projects

that could meet its requirements for stimulating the economy, and there’s no more ideal project to stimulate the economy than shipbuilding. And the *Sikuliaq* was really ready to go.” It was built by Marinette Marine Corp. in Wisconsin.

After additional outfitting and crew training, the *Sikuliaq* will head out next fall through the Great Lakes, travel down the Atlantic Coast, and move through the Panama Canal toward its home port of Seward, Alaska. It will start science operations in spring 2014.

Although it is substantially less powerful than the *Healy*, which can bust through 1.5 meters of first-year ice at a speed of 3 knots, the *Sikuliaq* is more versatile, Hawkins says. The ship is expected to spend between 60% and 80% of its time in open water, so planners traded a heavy hull and extra propulsion for an antiroll tank. “The community told us that one of the main criteria was to be as stable as possible,”

Whitledge says. That feature will likely be appreciated by any scientist or crew member who has struggled to deploy tow nets, water samplers, or drill cores from the rolling deck of a ship in high seas.

The new vessel will be a part of the consortium of research vessels known as the University-National Oceanographic Laboratory System (UNOLS). UNOLS vessels have year-round crews, giving them the chance to become intimately familiar with the ship’s features. “*Healy* is a great ship, but [the Coast Guard] does change everybody” on a rotating basis, Grebmeier says. “It really is nice for those of us who have been going to sea for 30 years to know who we’re going” to be sailing with.

—CAROLYN GRAMLING



On deck. The *Sikuliaq* rolls out toward a dock on Wisconsin’s Menominee River in preparation for its 13 October launch.

Alpha Helix was too small to accommodate increasingly large oceanographic mooring systems and other sophisticated science equipment; it could also only house 15 scientists at most. (The *Sikuliaq* can support 24.) “Only after the *Healy* came online in 2000 was there something that people could identify as a ship meant to do science,” Whitledge says. And as a Coast Guard vessel, the *Healy* must take on search-and-rescue missions as well as enforcing U.S. laws and international treaties.

Attempts to build an ice-strengthened vessel for Arctic research date to the 1970s, Whitledge says, but funding was scarce and exploring phenomena in the tropical and temperate zones was seen as a higher scientific priority. Work on designing the ship



Aftershocks in the Courtroom

An Italian judge will soon decide whether 30 people died because seven experts downplayed the risk of a major earthquake in L'Aquila in 2009

L'AQUILA, ITALY—"Stay calm. See you tomorrow morning." Those were the last words that Linda Giugno heard from her brother Luigi, at about 1 a.m. on 6 April 2009. Both lived in the central Italian town of L'Aquila, and Linda had phoned Luigi, a forester, because she was frightened by the latest in a long series of small- and medium-sized tremors that had shaken the town over the previous 3 months. Luigi said he didn't think there was any danger, and that there was no need for him to wake up his wife, who was due to give birth later that day, and their 2-year-old son.

A little more than 2 hours later, at 3:32 a.m., L'Aquila was struck by an earthquake with a moment magnitude of 6.3. Luigi, his wife, son, and unborn daughter were crushed to death as the 18th century building in which they lived collapsed—four of the quake's more than 300 fatalities.

Linda Giugno told her awful tale from the witness stand in a packed, silent courtroom here in October 2011—one of the first of many moving testimonies in a controversial manslaughter trial that has gripped L'Aquila

and scientists around the world.

On trial are seven men—four scientists, two engineers, and a government official—who participated in a meeting of an expert panel of Italy's Civil Protection Department (DPC) known as the National Commission for the Forecast and Prevention of Major Risks, which met on 31 March 2009 in L'Aquila to assess the ongoing series of tremors.

After the group adjourned, two members gave a press conference, accompanied by local officials. On that occasion, prosecutors say, they gave L'Aquila's inhabitants the mistaken impression that they had nothing to fear, and as a result, some people who would otherwise have fled their homes during subsequent tremors stayed inside—and were killed on 6 April. Indeed, when the prosecutor asked Linda Giugno why her brother was so sure there would be no destructive earthquake, her answer was clear: Experts quoted on TV news reports had said that there would be no tremors stronger than those already experienced.

The trial in L'Aquila has drawn huge international attention, as well as outrage and pro-

tests. In 2010, more than 4000 scientists from Italy and around the world signed an open letter to Italian President Giorgio Napolitano, calling the allegations "unfounded," because there was no way the commission could reliably have predicted an earthquake. Alan Leshner, CEO of AAAS (the publisher of *Science*) called the indictments "unfair and naïve" in a 2010 letter to Napolitano.

Yet as the trial unfolded here over the past year, a more complex picture has emerged. Prosecutors didn't charge commission members with failing to predict the earthquake but with conducting a hasty, superficial risk assessment and presenting incomplete, falsely reassuring findings to the public. They have argued in court that the many tremors that L'Aquila experienced in the preceding months did provide at least some clues about a heightened risk.

Meanwhile, a recorded telephone conversation made public halfway through the trial has suggested that the commission was convened with the explicit goal of reassuring the public and raised the question of whether the scientists were used—or allowed themselves to be used—to bring calm to a jittery town.

The trial is now in its final weeks; more than 100 witnesses have testified, including

Deadly toll. L'Aquila's 2009 earthquake killed 309 people and ruined the city's Medieval center.

geophysicists, engineers, public officials, psychologists, an anthropologist, as well as many friends and relatives of the victims. On 24 and 25 September, the prosecution presented its closing arguments in speeches totaling about 13 hours and asked for 4-year prison sentences for each of the seven defendants; this week, the defendants' lawyers were scheduled to start delivering their closing arguments. Finally, it will be up to a single judge, 43-year-old Marco Billi, to decide. His verdict is due by 23 October.

Tense and nervous

L'Aquila, the capital of the Abruzzo region, is in one of Italy's most seismically active areas. It lies practically on top of a fault that forms part of a larger system following the Apennine mountain chain for most of the length of the country. The town was struck by major earthquakes in 1349, 1461, and 1703—the latter the most lethal one, killing an estimated 2500 people. In 1985 and 1995, L'Aquila experienced so-called swarms, large numbers of fairly small tremors taking place over several weeks. They caused nervousness—but no major shock occurred.

Another swarm took place in the first few months of 2009, with tremors gradually becoming more frequent and more powerful (see graphic). They made the townsfolk increasingly tense and nervous, says geologist Antonio Moretti of the University of L'Aquila. That tension, he says, was com-

pounded by the predictions of Gioacchino Giuliani, a technician at the National Institute of Nuclear Physics near L'Aquila.

Giuliani says he can predict earthquakes by measuring increased emissions of radon gas from Earth—a theory that has been under investigation for decades but that most seismologists dismiss. Giuliani reportedly predicted that a strong tremor would strike the town of Sulmona, an hour's drive southeast of L'Aquila, on 29 March. The prediction triggered panic but it was wrong, and on 31 March, Giuliani was reported to the police for issuing an unjustified alarm, lead-



House of justice. With L'Aquila's old courthouse heavily damaged, the trial is being held in this makeshift building.

ing him to stop making public pronouncements on earthquakes.

Against this backdrop, the local magnitude of the tremors increased abruptly to 4.1 on 30 March, and Guido Bertolaso, then head of DPC, decided to convene the Major Risks Commission. Normally, the commis-

sion meets in Rome, but this time Bertolaso asked the group to travel to L'Aquila. The meeting's aim, according to a DPC press release issued on 30 March, was to "provide the citizens of Abruzzo all the information available to the scientific community on the seismic activity of the last few weeks."

Just ahead of the meeting, one of the commission members had already sounded very reassuring notes in an interview with local television station TV Uno. Bernardo De Bernardinis, then-deputy head of DPC and a hydraulic engineer, had said that the tremors posed "no danger" and that "the scientific community continues to confirm to me that in fact it is a favorable situation." The ongoing tremors helped discharge energy from the fault, De Bernardinis explained. Trial witnesses later said this was particularly reassuring because it suggested the danger decreased with each tremor. When the interviewer suggested that people could relax by pouring themselves "a good glass of wine," De Bernardinis replied "absolutely," and recommended a good Montepulciano.

The meeting itself kicked off at about 6:30 p.m. at the headquarters of Abruzzo's regional government and finished within an hour. Afterward, De Bernardinis gave a press conference along with the commission's then-vice-president, volcanologist Franco Barberi of the University of Rome (Roma Tre). They were joined by two officials who had attended the meeting:

The Seven Defendants

MEMBERS OF ITALY'S NATIONAL COMMISSION FOR THE FORECAST AND PREVENTION OF MAJOR RISKS IN 2009



Franco Barberi. Volcanologist at the University of Rome (Roma Tre), the commission's then-vice-president. Said during the meeting that the magnitude of tremors is very unlikely to increase in a swarm.



Enzo Boschi. Then-president of Italy's National Institute of Geophysics and Volcanology (INGV) and the country's most prominent geophysicist. Said the seismic swarm provided no signal of an impending major earthquake.



Gian Michele Calvi. Seismic engineer at the University of Pavia and president of the European Centre for Training and Research in Earthquake Engineering. Said future tremors shouldn't seriously damage buildings.



Claudio Eva. Seismologist at the University of Genova.



Bernardo De Bernardinis. Hydraulic engineer, then-deputy head of Italy's Civil Protection Department (DPC). Said minor tremors were "favorable" and suggested relaxing with a glass of Montepulciano.



Mauro Dolce. Seismic engineer, director of DPC's seismic risk office. Produced the meeting's official minutes.



Giulio Selvaggi. Seismologist at INGV and director of the National Earthquake Centre until he resigned in June of this year. Insists he was not a member of the commission but simply accompanied Boschi.

L'Aquila Mayor Massimo Cialente and the regional councilor responsible for civil protection, Daniela Stati.

The tenor of the statements they made, as reported in newspaper articles and television reports, was: Stay calm; it's not possible to predict earthquakes, but we don't expect a major quake is on the way. The newspaper *Il Tempo* reported De Bernardinis as saying that an increase in the magnitude of the tremors was not expected, while TV network Abruzzo24ore quoted Cialente as saying that "there should be absolutely no risk" of substantial damage to buildings.

Traditionally, prosecutors argued, people in L'Aquila had been trained by their parents to leave their homes as soon as they felt the ground shake, to avoid the effects of any further, potentially larger, tremors. That was what happened on the day before the meeting, when the magnitude-4.1 event happened; many people gathered near the castle or in one of the town's squares until they felt confident enough to go home. But the meeting of the Major Risks Commission changed many minds, contends the prosecution. "It was as if we were anesthetized, like someone had removed our primitive fear of the earthquake," the court was told by local surgeon Vincenzo Vittorini, whose family stayed inside the night of 5–6 April. "After that damned meeting, they instilled in us the idea that something terrible couldn't happen."

When the earthquake struck, with its epicenter little more than 3 kilometers from the town center, Vittorini lost his wife and daughter. The quake left 309 people dead, at least 1500 injured, and more than 65,000 were forced to leave their homes. More than 3 years later, the town seems frozen in time; most of the city center is abandoned, many of its streets still cordoned off, with some houses completely destroyed. Many older buildings are kept in a straitjacket of metal braces, while more modern apartment blocks have gaping holes that in some cases reveal pieces of furniture that are still standing.

A swarm's significance

With the original courthouse badly damaged in the quake, the trial is being held in a simple, bright blue building on an industrial estate several kilometers outside the town. Inside, there is barely enough room for the defendants and a small army of lawyers to sit, leaving standing room only for many friends and relatives of the victims and journalists.

For Fabio Picuti, the main prosecutor in the trial, the earthquake was the start of an unusual foray into a complex scientific field. Picuti is from L'Aquila and has spent most of his career investigating local organized crime, but he tells *Science* that he has studied the sci-

many of which were "at best scientifically useless" or, worse, "misleading."

Central to the prosecution's case is the swarm and what it implied about the risk of an impending quake. The scientists on the commission thought the swarm neither increased nor decreased the probability of a major earthquake. "A swarm, of whatever kind and of whatever duration, is never, and I underline never, a precursor of large seismic events," seismologist Giulio Selvaggi of the National Institute of Geophysics and Volcanology (INGV) told local newspaper *Il Centro* 3 weeks before the quake. (Selvaggi is one of three defendants who weren't officially on the commis-

sion but are regarded as members by the prosecution because they attended the 31 March meeting and had relevant expertise.) Barberi, who was the commission's vice-president, is quoted in a draft version of the meeting minutes as saying that "a seismic sequence doesn't forecast anything."

In their testimony, the defendants stuck to that opinion. Enzo Boschi,



In session. Public prosecutor Fabio Picuti (left) talks to Judge Marco Billi (right).

ence of the case extensively. He argues that had the commission members properly analyzed the seismic and other data at their disposal on 31 March 2009, and conveyed the results of that analysis accurately to the public, 30 of the victims of the earthquake would not have stayed indoors on the night of 5–6 April.

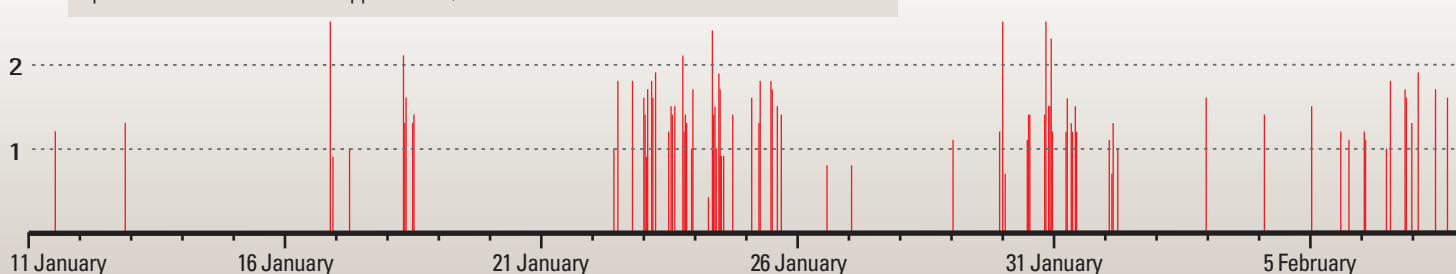
In his 509-page indictment, Picuti acknowledges that the experts were right to assert that predicting earthquakes is impossible, and that making buildings resistant is the best way to reduce risks. But he argues that these statements were of little use. He told the court that the minutes of the meeting in fact show the defendants to have made a series of "banal and self-contradictory" statements,

a geophysicist at the University of Bologna who for decades was the most prominent Italian geophysicist, told the court: "I refuse to admit that a seismic sequence, whether consisting of big or small tremors, can tell us a big earthquake is on its way." Boschi's lawyer, Marcello Melandri, adds that the experts did not undervalue the significance of the swarm. Melandri tells *Science* that the commission "did not reassure" during its meeting, adding that "it wasn't said that the earthquake wouldn't happen or that it would happen."

Picuti pointed out during his summing up that L'Aquila's 1461 and 1703 quakes were also preceded by foreshocks—and argued that the defendants knew this and should have taken it into consideration. "Why," he asked, "didn't another commission member

LOCAL MAGNITUDE

Trail of tremors. L'Aquila had experienced hundreds of minor quakes in early 2009. A key question in the trial: Did they suggest a big one might be coming? (The timing of individual spikes is based on GMT and is approximate.)



CREDITS (TOP TO BOTTOM): TIZIANA FABI/AFP/GETTY IMAGES; SOURCE: NATIONAL INSTITUTE OF GEOPHYSICS AND VOLCANOLOGY

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say: ‘No, Professor Barberi, we can’t make such a definite statement; let’s instead talk in terms of probability—that very rarely a seismic swarm can evolve into a strong tremor?’ If this had been written in the minutes, I certainly wouldn’t be spending my time here discussing this.”

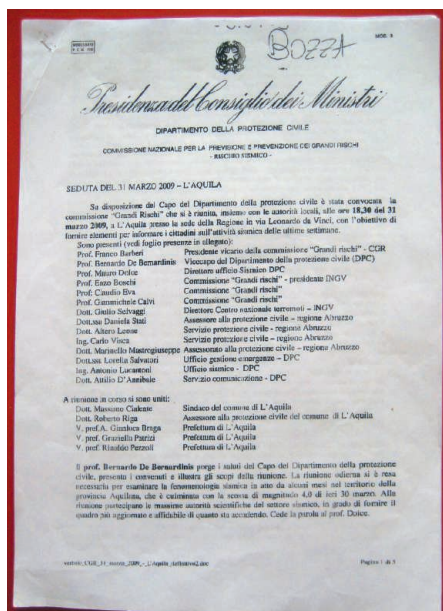
As Picuti also pointed out in the courtroom, the defendants’ position appears to differ from that of the International Commission on Earthquake Forecasting for Civil Protection (ICEF), which DPC set up in the wake of the L’Aquila quake to review the state of earthquake forecasting. In its report, issued in May 2011, ICEF said the occurrence of small or medium tremors does tend to increase chances of a large quake in the near future, even if the absolute probability remains low.

ICEF Chair Thomas Jordan, an earth scientist at the University of Southern California in Los Angeles, tells *Science* that the idea that swarms tell us nothing at all “is not quite right.” Many swarms do not lead to main shocks, but a few do, he says. “The frequency of main shocks is greater during swarm activity than it is without swarms,” he explains. “That’s where you get the notion that there is a probability increase.”

Apparent inconsistencies

Picuti also pounced on apparent inconsistencies in the commission’s assessment. One was a statement made during the meeting by Boschi. According to the meeting’s draft minutes, Boschi had said that the “periods of return [of major earthquakes in Abruzzo] are on the order of 2–3000 years. ... It is improbable that in the short term there will be a tremor like that of 1703, even if it can’t be ruled out absolutely.” Yet Boschi co-authored a 1995 study that estimated the probability of an earthquake of at least 5.9 in magnitude occurring in the L’Aquila area before 2015 at 1—in other words, it was certain to happen. “The head of Italy’s seismologists said [in the meeting] that it was improbable that there would be a major earthquake,” Picuti told the court. “It’s a shame he didn’t also inform them of his own study.”

Also under the prosecutor’s spotlight was



Risk assessment. Draft minutes of the 31 March 2009 meeting.

a statement made by Barberi during the meeting. According to the draft minutes, he said tremors within a swarm tend to have the same magnitude, “and it is very improbable that in the same swarm the magnitude will increase.” But Christian Del Pinto, a seismologist who attended the meeting as an observer, pointed out in testimony during the trial that the magnitude of the tremors had already jumped up—on 30 March, the very day before the meeting. It was therefore wrong to rule out further sudden rises in magnitude, Del Pinto said. Picuti told the court that Del Pinto’s observation was “dramatically important,” because that phrase, reported by the press, led people to their deaths. “Hence the judgment of guilt,” he said.

Barberi’s lawyer, Francesco Petrelli, says he can’t address Barberi’s comment before making his final arguments in court, which he was due to do as *Science* went to press. But he says the minutes don’t provide a word-by-word account of what was said in the meeting, and that “you have to read the text in its entirety and not in its single phrases.”

The public prosecutor also tackled what was perhaps the most controversial statement made by a member of the Major Risks Commission. In his now-infamous comments before the meeting, De Bernardinis said that

the swarm was actually “a favorable situation” because it caused a “continuous discharge of energy,” implying that it decreased the risk of a major quake. Other members of the commission told the court that this idea was not correct. Selvaggi, for example, described it as “a bit like an urban legend,” because the energy released by smaller tremors is insignificant compared to that given off in a damaging earthquake. But Picuti argued that the commission’s experts effectively sanctioned the notion when Barberi asked the other scientists for their opinion on this specific point. Based on the draft minutes, Picuti told the court that “none of them said a word.”

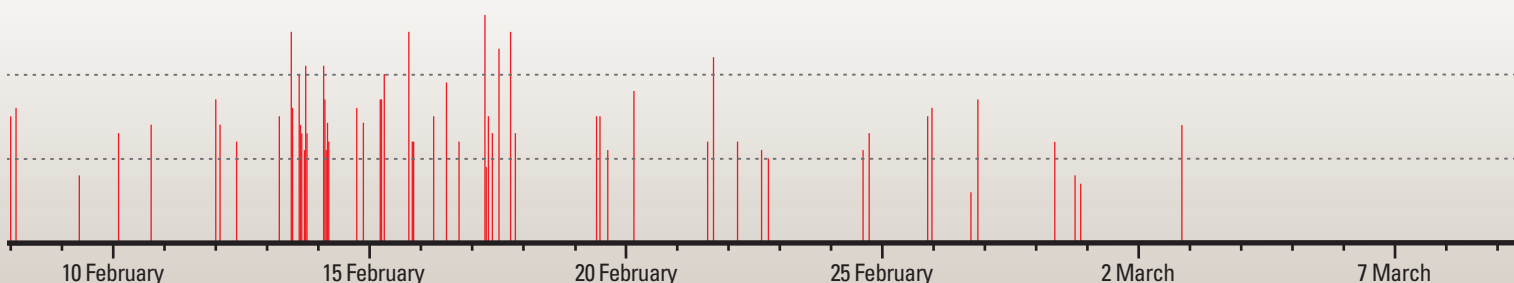
Chorus without soloists

In the course of the trial, new evidence also shed light on why the meeting was held in the first place—and played a role in attempts to shift the blame.

In January, *La Repubblica* released the bombshell recording of a telephone call made the day before the commission’s meeting. In it, then-civil protection head Bertolaso tells councilor Stati that he was convening the commission “not because we are frightened and worried” by the swarm but because “we want to reassure the public.” Bertolaso described the meeting as “more of a media operation.”

Boschi told the court that all Bertolaso apparently wanted to hear from the commission was that earthquakes can’t be predicted. “I imagined something more in-depth” from the meeting, Boschi told the court. The judge asked Boschi why he hadn’t objected to the discussion’s narrow scope. “For me, the head of the situation is the head of the civil protection,” Boschi replied, “and if he asks me to say this and that, I will say it.”

Moretti, the L’Aquila geologist, believes the mounting tension in the town forced Bertolaso to try to calm the public, and that the scientists were therefore constrained to make reassuring statements. “The scientists were forced towards an evidently mistaken decision and then abandoned,” he says. But Paolo Scandone, a geologist at the University of Pisa and a commission member in the 1980s, believes that the panel should have



insisted on sticking to the science. “The scientists perhaps weren’t lucid enough to say no to the administrators,” he says.

Similarly, opinions vary on where the responsibility lies for De Bernardinis’s controversial comments. Melandri tells *Science* that De Bernardinis’s comments were “his words” and not those of the commission. “The prosecutor has not distinguished between the different commission members,” Melandri says.

But Picuti argued that De Bernardinis reflected the position of the commission as a whole, describing the commission as “a chorus without soloists, an organism that speaks with a single voice.” De Bernardinis’s words “correspond exactly” with what was said during the meeting, Picuti told the court. “I realized during the course of the trial that De Bernardinis is the victim, the victim of the seismologists,” he said.

In his own testimony, De Bernardinis told the court that had the other commission members given him different advice about the possibility of a major quake, he would have taken action. “If they had said to me that the risk had increased,” he said, “I would have called Bertolaso [the civil protection department’s head] straightaway.”

Fonts of true knowledge

Even if the commission’s statements were wrong or misleading, for Judge Billi to convict the defendants of manslaughter, he must be satisfied that there was a direct causal link between their conduct and the victims’ decision to stay indoors on the night of 5–6 April 2009. That’s why a minor battle in the trial focused on the evidence for such a causal relationship. Testifying for the defense, neurologist Stefano Cappa of San Raffaele Hospital in Milan said that a direct link is impossible to prove because press reports and minutes relaying the commission’s conclusions “typically transmitted information that was ambiguous, generic and nonspecific.”

The prosecution brought in Antonello Cicciozzi, an anthropologist at the University

of L’Aquila, who argued in a written report that to the townspeople, the commission was made up of “maximum scientific authorities” and fonts of “true knowledge” not available to other people. Maurizio Cora, a lawyer who lost his wife and two daughters when their house collapsed, agreed. He told the court that he and his family awaited the statements of the commission “like manna” from heaven; on the evening of 5 April, together they “reasoned” on the basis of the commission’s statements that there would be no more powerful tremors than those already experienced. Reassured, they went to bed.

If Billi does find the defendants guilty, there will almost certainly be an appeal, which, with two or even three stages, could last up to 6 years, according to Fabio Alessandrini, a lawyer representing relatives and friends of the victims seeking damages. Given their different roles, only some defendants may be found guilty, Alessandrini says, and sentences may vary. Fines, which would probably be paid by the state rather than the defendants, could amount to tens of millions of euros, Alessandrini says. Bertolaso is now being investigated separately for manslaughter because of his role.

Jordan, the chair of the earthquake forecasting commission ICEF, does not believe anybody is guilty of manslaughter. De Bernardinis, he says, “made statements that were scientifically incorrect,” but he argues that he and his colleagues were engaged in a difficult balancing act—to communicate subtleties about changing seismic risk while trying to counter baseless predictions. “I think with hindsight they didn’t get that balance right, but I know from personal experience that it’s very tricky in those situations to say the right kind of things.”

Willy Aspinall, a professor of natural hazards and risk science at the University of Bristol in the United Kingdom, is more critical. He says the commission was hindered by an overcritical view of earthquake prediction that currently dominates the field. Past

failures to predict earthquakes have resulted in “mainstream seismology setting its face against any idea of prediction,” to the extent, Aspinall says, that many in the field also oppose the use of the less ambitious probabilistic forecasting. “Unfortunately, the experts thought it was evacuate or nothing,” he maintains. “In L’Aquila, people are used to sleeping in cars, and they shouldn’t have been dissuaded from that in my view.”

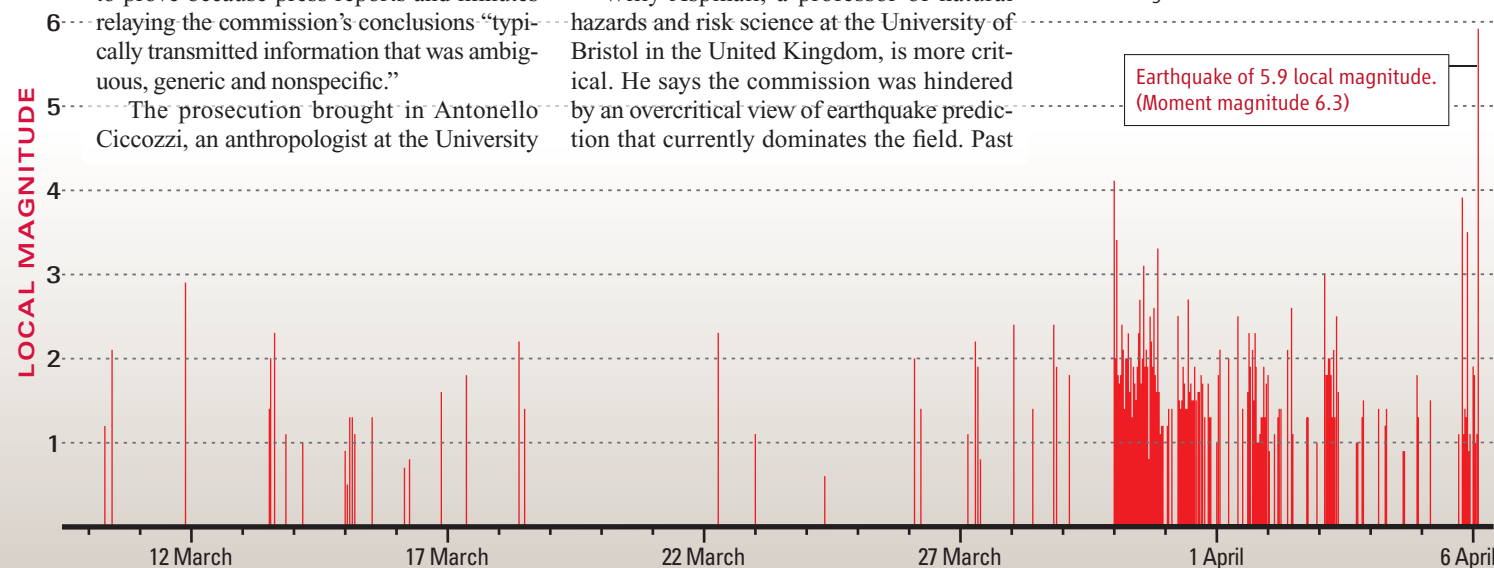
But Aspinall worries about the effect that a guilty verdict could have on scientific advice concerning natural hazards. He was chief scientist at the Volcano Observatory for the Caribbean island of Montserrat when an eruption killed 19 people in 1997. The enquiry into the deaths didn’t result in a criminal trial, but “the upshot is that the most sensible and best informed scientists are shying away” from giving advice on the island, he says. “If the L’Aquila scientists are found guilty,” he reckons, “we could end up with the charlatans and the mavericks.”

The best way to avoid such problems in the future, Jordan says, is to clearly delineate the role of the scientists and that of authorities responsible for civil protection. Experts should provide “carefully constructed probabilistic statements” regarding the risk, he says, which decision-makers would then use to choose the best course of action.

Vittorini, the surgeon who lost his wife and daughter, says he isn’t looking for the indicted to end up in prison either. The trial was “not a witch hunt,” he says. Its aim was to find out what mistakes were made and who was responsible, so that perhaps a similar tragedy can be prevented. “We need to change the mentality,” he says. “We need to make sure that people don’t [look to] reassure.”

—EDWIN CARTLIDGE

Edwin Cartlidge is a science writer in Rome.



Deadly toll. L'Aquila's 2009 earthquake killed 309 people and ruined the city's Medieval center.

geophysicists, engineers, public officials, psychologists, an anthropologist, as well as many friends and relatives of the victims. On 24 and 25 September, the prosecution presented its closing arguments in speeches totaling about 13 hours and asked for 4-year prison sentences for each of the seven defendants; this week, the defendants' lawyers were scheduled to start delivering their closing arguments. Finally, it will be up to a single judge, 43-year-old Marco Billi, to decide. His verdict is due by 23 October.

Tense and nervous

L'Aquila, the capital of the Abruzzo region, is in one of Italy's most seismically active areas. It lies practically on top of a fault that forms part of a larger system following the Apennine mountain chain for most of the length of the country. The town was struck by major earthquakes in 1349, 1461, and 1703—the latter the most lethal one, killing an estimated 2500 people. In 1985 and 1995, L'Aquila experienced so-called swarms, large numbers of fairly small tremors taking place over several weeks. They caused nervousness—but no major shock occurred.

Another swarm took place in the first few months of 2009, with tremors gradually becoming more frequent and more powerful (see graphic). They made the townsfolk increasingly tense and nervous, says geologist Antonio Moretti of the University of L'Aquila. That tension, he says, was com-

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House of justice. With L'Aquila's old courthouse heavily damaged, the trial is being held in this makeshift building.

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Just ahead of the meeting, one of the commission members had already sounded very reassuring notes in an interview with local television station TV Uno. Bernardo De Bernardinis, then-deputy head of DPC and a hydraulic engineer, had said that the tremors posed "no danger" and that "the scientific community continues to confirm to me that in fact it is a favorable situation." The ongoing tremors helped discharge energy from the fault, De Bernardinis explained. Trial witnesses later said this was particularly reassuring because it suggested the danger decreased with each tremor. When the interviewer suggested that people could relax by pouring themselves "a good glass of wine," De Bernardinis replied "absolutely," and recommended a good Montepulciano.

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HUMAN EVOLUTION

Turning Back the Clock: Slowing the Pace of Prehistory

New work suggests that mutations arise more slowly in humans than previously thought, raising questions about the timetable of evolutionary events

Timing is everything, especially in human evolution. For the past 45 years, researchers have used the number of mutations in DNA like a molecular clock to date key chapters in the human evolutionary story, such as the dawn of humankind millions of years ago and the exodus of modern humans from Africa in the past 100,000 years.

Now it seems that the molecular clock ticks more slowly than anyone had thought, and many dates may need to be adjusted. Over the past 3 years, researchers have used new methods to sequence whole human genomes, allowing them to measure directly, for the first time, the average rate at which new mutations arise in a newborn baby. Most of these studies conclude that the mutation rate in humans today is roughly half the rate that has been used in many evolutionary studies since 2000. "Together, these papers make a convincing case that the human sequence mutation rate is substantially less than the one previously used," says Harvard University population geneticist David Reich, co-author of one recent study. "As a result, genetic estimates of dates for ancient events are going to be older than previously reported."

The question now is how much older? Three new studies have taken a stab at providing an answer, trying to apply the slower mutation rates to major events in human evolution. In the past month, they have published a range of dates that sometimes fit with evidence from the fossil record—and that are sometimes way off, particularly for events further back in time. Some researchers suggest that the molecular clock may not

keep steady time across primates, and that it also has slowed over millions of years. This is confusing researchers who rely on those rates to study genetic diseases and the evolution of humans and primates.

"The mutation rates are so up in air," said paleogeneticist Svante Pääbo of the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany, in August, when his team published big margins of error—from 170,000 to 700,000 years ago—for the date when our ancestors split from Neandertals and their close cousins, the Denisovans. As a result, the timing of some events in human origins is now "very murky," says paleoanthropologist Chris Stringer of the Natural History Museum in London. The ambiguity in the mutation rate affects a host of evolutionary and disease-related analyses, says paleoanthropologist John Hawks of the University of Wisconsin, Madison: "We can't figure out how things happened if we don't know when they happened."

Fossil time

For the past 15 years, researchers have estimated the speed of the molecular clock by counting the mutational differences between humans and primates in matching segments of DNA, then using different species' first appearances in the fossil record to estimate how long it took those mutations to accumulate. For example, the fossils of the oldest known orangutan ancestor are about 13 million years old, so DNA differences between humans and orangutans had about that long to accumulate. By doing similar calculations in many segments of DNA in various

Time warp. The molecular clock used to date events in our prehistory may run more slowly than had been thought, and at variable speeds.

primates, researchers calculated an average rate of about one mutation per billion base pairs per year for humans and other apes.

When researchers plugged this rate into their equations, most got dates between 4 million and 6 million years ago for the split between the ancestors of humans and chimps. That dovetails pretty well with fossils identified as the earliest known hominins, or members of the human family, such as *Sahelanthropus*, which lived 6 million to 7 million years ago; *Orrorin*, which lived 6 million years ago; and *Ardipithecus*, which lived 4.4 million to 5.8 million years ago (*Science*, 15 February 2002, p. 1214).

As the rate became widely accepted, researchers used it to date milestones such as when the first modern humans migrated out of Africa—less than 70,000 years ago—and when they parted ways with Neandertals and Denisovans.

But this method of calculating the mutation rate has drawbacks. For starters, it assumes that the fossil dates accurately record the first appearance of a species, but that can change with a new find. Second, there are no fossils of our closest living relatives: chimps and gorillas. Third, the method assumes that species split at the same time as their genes diverged, but in fact, genetic separation can be millions of years earlier than species divergence. Finally, the method assumes that mutation rates are similar across apes, although factors such as generation time—the average number of years between generations—affect the rate.

Online

sciencemag.org

Podcast interview
with Ann Gibbons
(http://scim.ag/pod_6104).

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	Fossils	Old Mutation Rate	New Mutation Rate
Human-orangutan split	9 million–13 million years ago (<i>Sivapithecus</i>)	13 million–14 million years ago	34 million–46 million years ago
Human-chimp split	4.1 million–7 million years ago (<i>Sahelanthropus</i> , <i>Orrorin</i> , <i>Ardipithecus</i> , and <i>Australopithecus</i>)	4 million–7 million years ago	8 million–10 million years ago
Human-Neandertal split	350,000–600,000 years ago (<i>Homo heidelbergensis</i>) 200,000 years ago (Neandertals)	250,000–350,000 years ago	400,000–600,000 years ago
Out-of-Africa migration	125,000–80,000 years ago (archaic <i>Homo sapiens</i>)	Less than 70,000 years ago	90,000–130,000 years ago
Seeking synchronicity. Some dates derived using the new, slower mutation rate match fossils—and some don't.		Match between fossils and molecular date	
		Mismatch between fossils and molecular date	

The first sign that something was amiss came in 2003, when a study tracking genes that cause hemophilia, muscular dystrophy, and other diseases in parents and children found slower mutation rates than expected.

With the recent advent of high-throughput sequencing methods, geneticists finally have been able to sequence enough whole genomes to calculate directly the number of mutations between trios of two parents and their child in large numbers of families. Eight studies in the past 3 years (and the 2003 study) have estimated a slower mutation rate, according to a review published online on 11 September in *Nature Reviews Genetics* by geneticists Aylwyn Scally and Richard Durbin of the Wellcome Trust Sanger Institute in Hinxton, U.K. Several studies sequenced the complete genome, such as one in August that measured the mutation rate in 78 trios of Icelandic parents and children. Other papers report the mutation rate in disease-causing genes, including one study that sequenced genes in 14,000 individuals.

The Icelandic study found that on average, every newborn baby has 36 spontaneous new mutations, those not inherited from either parent. These rare, so-called *de novo* mutations are just a tiny proportion of the 3 billion nucleotide bases in the genome, but this is the source of all variation in humans. It is the raw material for all human evolution—for better or worse, because most new mutations are deleterious. “All of the diversity in the human genome was once upon a time *de novo* mutation,” says geneticist Kári Stefánsson of deCODE Genetics in Reykjavík, co-author of two new studies in Icelanders. “We have evolved through these new mutations.”

Remarkably, all the studies got about

the same rate: 1.2×10^{-8} mutations per generation at any given nucleotide site. That’s about 1 in 2.4 billion mutations per site per year (assuming an average generation time of 29 years)—and that’s less than half of the old, fossil-calibrated rate. “It really does look good; all the studies are pointing in the same direction, averaged over many individuals,” says evolutionary biologist Michael Lynch of Indiana University, Bloomington, co-author of one of the studies on the mutation rate.

If geneticists know the average rate, they can measure whether it is higher in genes that play a part in diseases such as autism, schizophrenia, or prostate cancer that are on the rise, Stefánsson says. And then they can begin to tease out what factors might change the rate in those genes.

Pushing back time

A slower-ticking molecular clock also has major implications for evolution. For example, the slow clock suggests that the ancestors of modern humans and Neandertals diverged about 400,000 to 600,000 years ago, rather than 272,000 to 435,000 years ago. This fits nicely with fossils of *Homo heidelbergensis*, which date between 350,000 and 600,000 years ago and are thought to be ancestral to Neandertals. Scally and Durbin also revised the dates for modern human evolution, such as pushing back the timing of a dramatic population bottleneck from 100,000 to 120,000 (rather than 60,000 to 80,000) years ago, and the emergence of modern humans out of Africa to 90,000 to 130,000 (rather than less than 70,000) years ago.

Researchers who study fossils and stone tools older than 100,000 years in the Middle East and North Africa say this boosts the

the Neandertal dates seem on the early side to Stringer: “We probably do not have clear Neandertal features before about 400,000 years ago—or at a real push, 500,000 years,” he says. “So, I do not see good reason—yet—for major revision” of the dates for Neandertal and modern human origins.

What’s more, the new rate slows the pace of evolution in apes to a downright crawl (see table). It puts the split of humans and chimpanzees, for example, at between 8.3 million years ago and 10.1 million years ago—far too early, given current fossil dates. The split of the lineages leading to orangutans and the African apes, including humans, goes back to 34 million to 46 million years ago, Reich says. “A human-orangutan split at 40 million years is absolutely crazy,” says paleoanthropologist David Begun of the University of Toronto, St. George, in Canada, who notes that fossils of likely orangutan ancestors date from 9 million to 13.9 million years ago.

Recognizing these problems, Scally and Durbin propose a fix: They endorse a 30-year-old idea that the mutation rate was faster early in primate evolution, then slowed in the African apes, and perhaps slowed even more in human evolution—the so-called hominoid slowdown. This idea is backed by evidence that as body size gets bigger in various mammals, their generation times get longer, slowing per-year mutation rates, Scally says. It’s an intriguing idea that leads to a better fit between fossils and Scally’s dates. “Some kind of slowdown certainly occurred in apes,” Reich says. But he cautions that “the magnitude of slowdown required to reconcile these dates is extreme.”

Indeed, a separate study recently challenged the link between bigger bodies and longer generation times. After a decade of

case that these ancient people might be ancestral to modern humans rather than a failed exodus out of Africa. But others who have tied the bottleneck or out-of-Africa migration to events such as a major volcanic eruption about 70,000 to 75,000 years ago are likely to be less pleased. “Some people will be happy with these rates, and some will not,” Hawks says.

Push back a little further in time, and the fit with fossils becomes strained. For example,

work recording the age of parents at the birth of 226 chimpanzees and 105 gorillas in the wild in Africa, Linda Vigilant of the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany, and an international team reported in August that gorillas have a shorter generation time—about 19 years—than their smaller human and chimp cousins, who average about 29 years (thanks to older fathers) and 25 years, respectively.

Vigilant's team, whose work was published in the *Proceedings of the National Academy of Sciences*, went a step further, multiplying their new generation times by the new, slower mutation rate to date events in evolution. Their result also pushes back the human-chimp split to 7 million to 13 million years ago, which just fits with the oldest fossils, Vigilant says. But those dates assume that generation times haven't changed in millions of years. "A big question is, what were generation times in the past?" Vigilant says.

Reich, meanwhile, is a co-author on yet another study that used a different method and got an intermediate rate. He says it's possible that the new methods aren't picking up all the mutations, and so could be getting an artificially slow rate. So he, Stefánsson, and graduate student James Sun of the Massachusetts Institute of Technology in Cambridge analyzed mutation rates in 2500 microsatellites—small pieces of DNA that vary in the number of times they repeat—in 85,000 people in Iceland. Microsatellites have a higher rate of mutation than DNA nucleotides, so it is easier to detect all their new mutations. The team came up with a way to convert the microsatellite mutation rates back to a base pair mutation rate, which they estimate at 1 in 1.2 billion to 1 in 2.0 billion per year.

That's also a better fit with fossils, putting the split between humans and chimpanzees at about 3.7 million to 6.6 million years ago. This would create a different kind of problem, however: The oldest fossils vying to be the earliest members of the human family, such as *Sahelanthropus* at 6 million to 7 million years ago, might be too old to be hominins. So no matter how researchers calculate the mutation rate directly, they can't accommodate all the fossil dates.

Rate adjustment

All these complicating factors show that researchers are still exploring what alters the mutation rate, and by how much. Yet another twist may be the age of fathers at conception. In a landmark study of 78 trios of parents and offspring in Iceland, Stefánsson and Augustine Kong, also of deCODE Genetics,

found that older fathers pass on more mutations to their offspring, because mistakes are made as aging DNA is copied to produce sperm over men's lifetimes. (Older mothers don't pass on more mutations, because eggs are all produced before birth.) The effect is large, causing two extra mutations per year of father's age. That means that 36-year-old dads pass on twice as many mutations as 20-year-old dads, according to a report in *Nature* in August. So an increase in the average age of fathers might have sped up the molecular clock. "It's difficult to extrapolate back like this," Stefánsson says. "You have to recognize that there are going to be flawed estimates."

Other researchers are trying to explore what affects the mutation rate by studying parts of the genome with rates that are much above or below the average. "There is a lot of variability in the rate at which DNA evolves from one part of the genome to another," says geneticist Katherine Pollard of the University of California, San Francisco, although she adds that such hot spots are such a small percentage of the chimp and human genomes that they should not affect the average mutation rate.

And in a forthcoming paper, Lynch and his colleagues show that big genomes and big populations both slow the mutation rate in model organisms such as bacteria, yeast,

plants, and fruit flies. This underscores how risky it is to assume that the mutation rate was constant in humans and apes, because so little is known about what might speed up or slow the clock over great sweeps of time. A bottleneck that cut the number of breeding adults to 10,000 before modern humans swept out of Africa would be expected to speed up the rate, Lynch says. But did the rapid expansion of modern human populations after the invention of agriculture then slow the rate? To be surer about extrapolating the rate across the apes, researchers need to calculate the mutation rate directly in chimp and gorilla parents and their offspring, as they have done for humans, Lynch says. But even when that work is done, the modern rate in these small, endangered ape populations may be faster than it was in larger ancestral populations.

Thus, although both geneticists and paleoanthropologists are attuned to the fact that the human mutation rate today is slower than previously believed, it's still unclear how much slower it is, and what that means for timing events in our past. For now, "the question remains open—what is the true value of the mutation rate," Reich says. And "to do evolutionary analysis, you want to calibrate our clock properly."

—ANN GIBBONS



Just in time? Different mutation rates make this 6-million to 7-million-year-old fossil of *Sahelanthropus* too young, too old, or just the right age to be an ancestor of humans.



LETTERS

edited by Jennifer Sills

Baits, Budget Cuts: A Deadly Mix

THE ILLEGAL USE OF POISON BAITS IS THE MOST IMPORTANT NON-natural factor in the the extinction of several European vertebrate megafauna over the previous two centuries. Yet the practice continues unabated today. Poison baits represent a serious threat to public health and a serious conservation problem for sustaining biodiversity at both European and global scales (1).



Bearded vulture with GPS transmitter.

Spain is home to important populations of several threatened vertebrate species. More than 8000 cases of illegal poisoning were reported in the period between 1990 and 2010, with victims including 53 bearded vultures (*Gypaetus barbatus*), 366 Egyptian vultures (*Neophron percnopterus*), 759 cinereous vultures (*Aegypius monachus*), 117

Spanish imperial eagles (*Aquila adalberti*), 2877 Eurasian griffon vultures (*Gyps fulvus*), 1981 red kites (*Milvus milvus*), 961 black kites (*Milvus migrans*), and 9 brown bears (*Ursus arctos*) (2–5). Several of these species are classified as endangered within the European Union (there remain only 170 pairs of bearded vultures, 323

Spanish imperial eagles, 1889 cinereous vultures, and 1900 Egyptian vultures). Moreover, Spain is home to more than 95% of all European avian scavengers and the world's entire Spanish imperial eagle population. Given this context, the damage to the conservation of European biodiversity caused by poisoning is considerable.

In light of the current economic crisis, the Spanish government has cut funding for research and development, and its Ministry of Agriculture, Food, and Environment has reduced investment by 31% with respect to 2011. As a result, research and conservation programs that can minimize the impact of illegal poisoning are at risk. Without the funds to monitor threatened species with satellite transmitters, to analyze animal carcasses found through this and other monitoring methods, and to continue with environmental education programs and research trap selectivity methods, illegal poisoning looms even larger. All the human and economic efforts of the past two decades could turn out to be futile and biodiversity be put at risk if research and conservation programs are paralyzed.

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Saving Vietnam's Wildlife Through Social Media

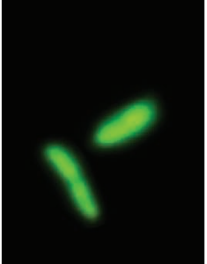
ALTHOUGH VIETNAM IS A GLOBALLY RECOGNIZED biodiversity hotspot with 59 new species discovered in 2010 alone (1), the state of wildlife conservation therein is a matter of serious international concern. Vietnam is a major consumer and exporter of wildlife, as well as a source and conduit for the illegal trade from Laos, Cambodia, and Myanmar to China (2). Over a 10-year period, Vietnamese authorities have confiscated over 180,000 wild animals destined for trade. It is estimated that this represented only 5 to 10% of the actual amount illegally traded (3), about half of which is consumed in the domestic

market (2). In fact, consuming wildlife has become the norm among Vietnamese. Half of Hanoi residents have used wild animal products (4), and almost a third of all Vietnamese have used bear bile to treat illness (5).

In a recent report, the World Wildlife Fund ranked Vietnam last of 23 countries in implementing its commitments to prevent the illegal trade of elephant, rhino, and tiger products through the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (6). A large share of the responsibility for the dire state of conservation can be attributed to Vietnamese society, which both drives wildlife consumption and behaves passively toward conservation-related scandals. This passivity is illustrated by the seeming acceptance of the

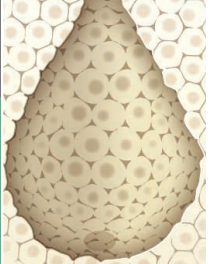
public toward incidents such as the purchase of rhino horn by a diplomat from the Vietnamese Embassy in South Africa (7) and the official—yet illegal—auctioning of confiscated tiger bone glue with approval from the Provincial People's Committee (8).

Social media offer a major tactical opportunity to hold public officials and citizens accountable, by galvanizing public opinion, applying public pressure, and therefore incentivizing improved conservation behavior. For example, in July, photos of a pregnant endangered douc (*Pygathrix cinerea*) being tortured and slaughtered in the presence of Vietnamese soldiers were posted on Facebook. The story grabbed the attention of readers and generated substantial public outcry (9). Such mass criticism over mistreatment of endangered



Less biomass below
the ocean floor

204



Cilia sense
the flow

206

animals had not been previously experienced in Vietnam. Under this public pressure, the government dismissed the three soldiers in an unprecedented military ruling (10).

This example demonstrates the potential power of social media to influence an immediate government response to a conservation crisis. Social media are also being used for longer-term objectives, such as curbing the consumption of shark fin in China (11) and pressuring retailers to stop selling shark fin products (12). Scientists and conservationists need to embrace the full potential of social media, as have other sectors of society, such as intelligence communities that use social media to predict riots or war outbreaks (13), or organizations striving to improve political self-expression and voter turnout (14). The social media can thus represent, combined with education, a very powerful tool for conservation.

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Making Science Education Relevant

FOR AS LONG AS I CAN REMEMBER, I HAVE been worried about our failure as a nation to grasp and apply basic scientific principles. I believe that the vast majority of attempts to teach science and engineering to the average student are doomed to failure for one simple reason: Unlike our educators, our students are not convinced that science and engineering are important. I think we need to shift our focus from "important science" to "science that's important to the public." To do so, we must rethink what material should be covered and how to best present it in our writing, publications, and courses.

I tried out this approach at the University of Virginia. I offered a course called Responsible Citizenship in a Technological Democracy for undergraduates with no science, math, or engineering background. I presented a series of public policy issues (especially ones for which the conventional wisdom is wrong) and introduced the science and engineering concepts needed to understand each one. Then the students used these concepts to analyze the issues. Topics included electric cars, the hydrogen economy, and paper cups, and scientific subjects such as statistics, risk, and precision versus accuracy (1). I found that students cared about getting public policy right. When confronted with evidence that conventional wisdom is wrong, they were motivated to understand the science and engineering concepts. Suddenly, they too considered science important.

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1. IEEE Central Virginia Section, Responsible Citizenship in a Technological Democracy (www.cvaieee.org/html/resp_citizen/responsible_citizenship.html).

CORRECTIONS AND CLARIFICATIONS

Perspectives: "Real fish attack simulated plankton" by W. L. Romey (7 September, p. 1181). The first sentence of paragraph five should have stated that the predatory fish studied by Ioannou *et al.* preferentially attack smaller, not larger, groups of simulated prey. Also, paragraphs four and five incorrectly stated that members of simulated groups of zooplankton in Ioannou *et al.*'s model always follow similar rules. Instead, each member of a group may have different movement rules due to evolution within the simulation.

TECHNICAL COMMENT ABSTRACTS

Comment on "Climatic Niche Shifts Are Rare Among Terrestrial Plant Invaders"

Bruce L. Webber, David C. Le Maitre, Darren J. Kriticos

Petitpierre *et al.* (Reports, 16 March 2012, p. 1344) conclude that niche shifts are rare for terrestrial plant invaders and that this justifies the use of correlative modeling to project species geographic ranges for biological invasions and climate change. We draw attention to the limitations of their conceptual assumptions and the importance of niche shifts excluded from their analyses.

Full text at <http://dx.doi.org/10.1126/science.1225980>

Response to Comment on "Climatic Niche Shifts Are Rare Among Terrestrial Plant Invaders"

Antoine Guisan, Blaise Petitpierre, Olivier Broennimann, Christoph Kueffer, Christophe Randin, Curtis Daehler

Webber *et al.* take a critical view of our findings that niche expansions are rare in plant invaders, arguing mainly that we did not include nonanalog climates in our analyses. Yet, their concerns include misunderstandings and go beyond the scope of our study, which was purposely restricted to analog climates. We further explain why our results remain robust to other factors of niche dynamics in the native range. We conclude that the implications of our findings remain valid for projections of niche models in analog climates and that projections in nonanalog climates should be undertaken with care.

Full text at <http://dx.doi.org/10.1126/science.1226051>

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the past 3 months or matters of general interest. Letters are not acknowledged upon receipt. Whether published in full or in part, Letters are subject to editing for clarity and space. Letters submitted, published, or posted elsewhere, in print or online, will be disqualified. To submit a Letter, go to www.submit2science.org.

Comment on “Climatic Niche Shifts Are Rare Among Terrestrial Plant Invaders”

Bruce L. Webber,^{1*†} David C. Le Maitre,^{2†} Darren J. Kriticos^{3†}

Petitpierre *et al.* (Reports, 16 March 2012, p. 1344) conclude that niche shifts are rare for terrestrial plant invaders and that this justifies the use of correlative modeling to project species geographic ranges for biological invasions and climate change. We draw attention to the limitations of their conceptual assumptions and the importance of niche shifts excluded from their analyses.

Ecological niche modeling for invasive species and species range shifts under climate change relies on the ability of the model to project robustly into novel climates (1). This, in turn, depends entirely on the extent to which a species' range, or part of its range, is defined by abiotic factors that can be revealed by inferential modeling techniques, or by other limiting factors that may not be detectable a priori (2). Petitpierre *et al.* (3) contend that only expansion to novel climates that are available in the native range (i.e., the available niche space) should be considered unequivocally as niche shifts, assuming that expansion into nonanalog climates [i.e., those beyond the native realized niche (Fig. 1)] is somehow different because it may represent the filling of a preadapted niche. We disagree with this thesis and counter that exaptation [as used in (4)] constrained by biotic interactions or dispersal limitation could just as easily explain niche expansion into available climatic space in the native range as it could expansion into nonanalog climates. At the heart of our disagreement are two different aspects of a species' Grinnellian realized niche: first, our ability to estimate it by different means, and second, its definition.

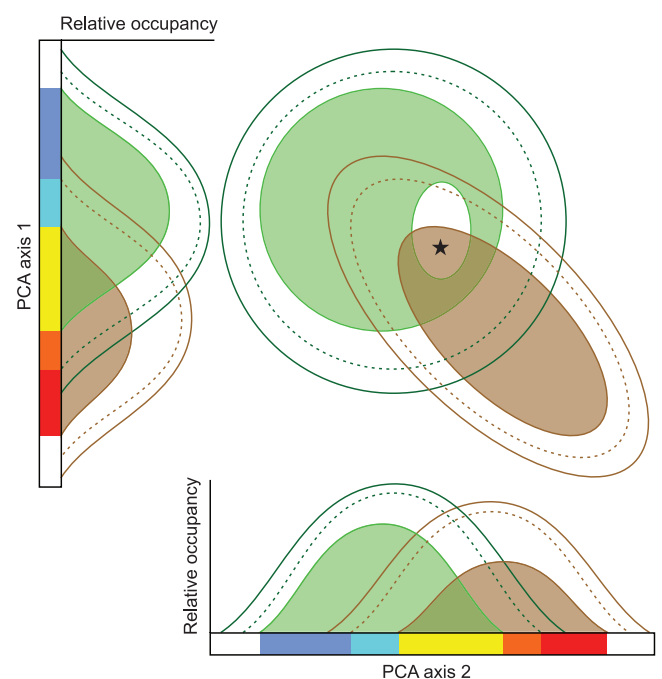
A recent review concluded that species' niches were remarkably stable across a variety of scales (5) but did not go on to conclude that climatic niche expansion was similarly limited. Within its native range, a species is likely to be constrained by both biotic and abiotic factors, and possibly by dispersal limitations (6, 7). When intro-

duced into a new region beyond its native range, it is sometimes the case that species appear to expand their realized climatic niche when assessed using modeling tools. Such expansion can arise when the new location has nonanalogous climates but where the values of the key climatic variables [raw or principal component analysis (PCA) transformed] still fall within the species' physiological limits. For example, a species that evolved in a Mediterranean climate may thrive in a subtropical climate that was not within or accessible to its native range. Using correlative species distribution models relating species distribution records to Bioclim or PCA-transformed climate variables, any subtropical climates should be outside of the climate space used to train the model and may not be correctly identified as suit-

able by a model trained on the native range data alone. From a practical perspective, this example may represent a realized niche expansion, and this possibility could be tested using mechanistic models, including data based on the native and alien ranges. The interpretation of this niche expansion is a somewhat separate issue. It could arise because the species was exapted to the novel climatic conditions—its ability to survive the stressful Mediterranean climatic conditions conferring on it an ability to tolerate subtropical ecosystems—or it could depend on other factors, such as the release from natural enemies (8) or a favorable habitat disturbance pattern. Irrespective of the mechanism, the point is that these estimates of niche shift represent an expansion only in relation to our ability to define the shift based on niche modeling tools [including the niche shift kernel method (3, 9)].

In arguing that a shift to only those climates that are available in the native range can unequivocally define evidence of niche expansion when occupied in the alien range, Petitpierre *et al.* are ignoring the ordinal nature of the PCA-transformed climate space and the ecological law of tolerance (10). One cannot limit the term “niche expansion” to the PCA space (and thus climate space) shared between the native and alien ranges (orange zone, Fig. 1) without also considering the more extreme PCA space occupied by the species (red zone, Fig. 1) as also constituting an expanded realized niche. Furthermore, by explicitly constraining their analyses to the former highly conservative component of niche expansion in

Fig. 1. Niche shifts for native (green circle) and alien (brown oval) realized niches of a hypothetical species as defined by two axes of a PCA of climatic variables. Relative occupancy of the realized niches is shown along each axis for the native (green shading) and alien (brown shading) realized niche. Full and 75th percentile lines (solid and dashed, respectively) are depicted for the entire range of conditions available in the native (green) and alien (brown) regions of study as defined for modeling purposes. Niche shift kernel analyses were conducted by Petitpierre *et al.* using the 75th percentile. Along the axes, “unfilling” (light blue), “stability” (yellow) and “expansion” (orange) regions as defined by Petitpierre *et al.* are shown alongside additional unfilling (blue) and expansion (red) in nonanalog climates that are not considered by their niche shift kernel technique. The star indicates niche shift to an unoccupied area within all possible combinations of the PCA values for the native realized niche. [Adapted from figure 10.2 in (13) with permission]



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PCA space (Fig. 1), Petitpierre *et al.* actively exclude a critical component of niche shifts when modeling species for invasions and with climate change. When introduced into a new location or with climate change, a species' niche can expand by both infilling into novel combinations of covariates within the range available to the species and by expansion into novel covariate values beyond those currently experienced (Fig. 1). We regard niche shifts relating to the former situation as more likely to be driven by geographic barriers and nonclimatic abiotic factors, whereas examples of the latter are more likely to be driven by changes in biotic interactions. Furthermore, elements of infilling (star, Fig. 1), as quantified by Petitpierre *et al.* in their figure 3, are arguably uninformative to understanding the potential niche of the species. Although we suggest that niche conservatism for invasive aliens will be the exception rather than the rule, without careful consideration of model performance in both analog and nonanalog (i.e., interpolation and extrapolation) climate space, it is impossible to assess the ecological plausibility of any model output.

It seems clear to us that the constrained niche shift kernel concept employed by Petitpierre *et al.* greatly diminished their ability to identify niche shifts for alien species (and native species) under climate change. The Petitpierre *et al.* study's concepts of unfilling and expansion may be useful in some contexts, but their limited concept of expansion does not allow them to exhaustively explore whether species niches appear to shift or change in nonanalog environments. Addressing potential niche shifts by alien or native species requires robust models that permit exploration of nonanalog climates (11). We are similarly concerned, therefore, that the subsequent ecological niche models of Petitpierre *et al.* for alien species were specifically constrained to avoid any extrapolation beyond the training domain (i.e., available niche space in the native range). We argue that their conclusion that ecological niche models are suitable "for the prediction [sic] of both biological invasions and responses to climate change" does not hold, and we find no support for any change in the caution (12) to be extremely wary when using correlative ecological niche models to project

potential distributions for alien species or with climate change.

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12 June 2012; accepted 17 August 2012

10.1126/science.1225980

Response to Comment on “Climatic Niche Shifts Are Rare Among Terrestrial Plant Invaders”

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Webber *et al.* take a critical view of our findings that niche expansions are rare in plant invaders, arguing mainly that we did not include nonanalog climates in our analyses. Yet, their concerns include misunderstandings and go beyond the scope of our study, which was purposely restricted to analog climates. We further explain why our results remain robust to other factors of niche dynamics in the native range. We conclude that the implications of our findings remain valid for projections of niche models in analog climates and that projections in nonanalog climates should be undertaken with care.

Webber *et al.* (1) take a critical view of our findings that niche expansions are rare in plant invaders when only climates available in both native and invaded ranges are considered (2). Their main point of contention is that we did not include in our analyses those climates in the invaded range that are not available in the native range (i.e., nonanalog climates). Webber *et al.* argue that niche shifts could occur in nonanalog climates and that they are important when making predictions with niche models. They also argue that other factors in the native range could explain these niche changes. However, their comments mix different issues, misinterpreting our findings and going far beyond the scope that we defined for our results and conclusions. We reply here to their main points.

First, we never concluded that a niche expansion—especially of the realized niche as Webber *et al.* argue—could not occur in nonanalog climates. Indeed, as we reported, our niche change quantification framework allows all situations to be quantified, including both analog and nonanalog situations, and we reported [(2) and its supporting online material] that niche analyses in total climate reveal more shift. We chose, however, not to report on analyses involving nonanalog situations in the main report because their interpretation remains highly speculative (3–5). There, only complementary experimental approaches could help determine whether the expansion is

caused by a real change of the fundamental niche or is due to preadaptation (6, 7), because the fundamental niche cannot be quantified using empirical distribution data (8). Indeed, when Webber *et al.* state, “Such expansion can arise when the new location has nonanalogous climates but where the values of the key climatic variables...still fall within the species’ physiological limits,” they give credit to our approach of not considering nonanalog environments, because what they describe is precisely the preadaptation situation just discussed. In this regard, their speculative case of a Mediterranean species invading tropical areas would also be an example of preadaptation. These nonanalog situations can indeed be quantified with our framework, but they cannot be ascertained as being an expansion of either the fundamental or realized niches because, without experiment, one cannot know whether the species would (e.g., preadaptation) or would not (e.g., outside the niche, dispersal limitations, or biotic exclusion) have occupied these environments if they had been available in the native range. Still, we acknowledge that these situations of niche filling in nonnative conditions are important when making spatial predictions and can be accommodated by fitting models with data from both native and invaded ranges (9, 10).

Second, we agree that dispersal limitation or biotic interactions can effectively result in geographic unfilling in the native range (11), but unfilling in geographic space does not translate into unfilling in climatic niche space. Furthermore, if native niche unfilling was occurring, we should indeed observe more niche expansion in the introduced range. More important, though, Webber *et al.* misunderstand our analyses and graphs when they write: “Furthermore, elements of infilling (star, Fig. 1), as quantified by Petitpierre *et al.* in their figure 3, are arguably uninformative to understanding the potential niche of the species,” because they compare two very different things. Whereas their figure 1

conceptually compares the niche of one species between ranges, our figure 3 is based on observations and represents the accumulation of events of (i) niche expansion versus stability and (ii) niche unfilling versus stability across all species, as a way of summarizing the extent of these phenomena in climatic space. Therefore, holes in the niche of a single species (their star) simply cannot be observed in our graphs because there is no way to represent the niches of individual species there. Another complication of their figure 1 is that the hole they represented in the two-dimensional (2D) niche (their star) is not represented in the corresponding 1D response curves (in the native range), resulting in a discrepancy between their interpretation of niche changes along the principal component analysis axes (their gradient from red to yellow to blue) and the real niche in 2D. Furthermore, in our work we deliberately did not use the term “infilling” because the situation they describe (star in their figure 1) is unlikely to persist in our quantitative framework (12), which uses a kernel density smoother that aims precisely at avoiding these ecologically unrealistic situations of “holes” within a species’ niche. This framework builds on previous work [e.g., (13)] and therefore represents the latest advance in multivariate niche quantification, thus accounting for a modern, probabilistic definition of the niche, including older concepts such as the law of tolerance.

Third, we agree that studies of nonanalog climates would be useful to forecast effects of climate change and biological invasions, but these cannot rely on current distribution data [(13) and below]. As developed above, we avoided nonanalog situations because these were recently shown (3, 4, 14) to be conditions where predictions by ecological niche models should not be made [see (14) and the “C” area in its figure 1]. Following these recommendations, and contrary to claims by Webber *et al.*, what our study truly demonstrated is that assessing climate change impact within the range of analogous climates is likely to be a valuable use of climate niche models. It is therefore curious to see the same reference (14) supporting our choice to exclude nonanalogous climates, also used by Webber *et al.* to question our conclusions.

To conclude, Webber *et al.* provide themselves the main conclusion to our reply when they write “their limited concept of expansion does not allow them to exhaustively explore whether species niches appear to shift or change in nonanalog environments.” We can only agree, as our study never pretended to address niche shifts in nonanalog situations. We further agree that complementary studies of niche changes in nonanalog situations would be particularly useful to assess the ability of niche models to project into nonanalog climates (in the future or in different ranges) and that these studies will need to rely on more mechanistic approaches based on experimental measurements, not only on empirical distribution data.

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Acknowledgments: A.G., B.P., and O.B. thank the Swiss National Centre for Competence in Research (NCCR) "Plant Survival in Agro-ecological Landscapes" for financial support.

25 June 2012; accepted 17 August 2012
10.1126/science.1226051

HISTORY OF SCIENCE

Heterodoxy and Its Discontents

Alex Wellerstein

The history of bad ideas is as interesting, and as important, as the history of good ones. Books on the histories of Creationism, eugenics, and Lysenkoism—to pick just a few famously bad ideas—have proven illuminating to those who want to know how science functions (or doesn't) on the margins and how it is co-opted into popular (and political) ends. Princeton historian of science Michael Gordin's *The Pseudoscience Wars* explores a lesser-known 20th-century movement, Velikovskyism, and uses this as a lens with which to understand the power of pseudoscience in an age where scientific authority and funding have never been higher.

Gordin observes anecdotally that the name Immanuel Velikovsky is essentially unknown to anyone under the age of fifty. (It was meaningless to me.) Nonetheless, there is a story

book of Exodus, the planet Jupiter ejected a massive comet that became trapped in a gravitational and electromagnetic interaction with Earth. For the next several decades, these interactions caused the supernatural events described in the Old Testament (for example, the “manna from heaven” were hydrocarbons rained down by the comet's tail), as well as similar catastrophes described in other religious traditions. Eventually the comet settled into a stable orbit and as such became the planet we know as Venus.

For evidence of these extraordinary claims, Velikovsky cited meticulously correlated myths from ancient history (much of which he had redated according to his own chronology), as well as his own idiosyncratic electromagnetic theory of gravity and a distinctly Freudian approach to the study of history. Moreover, Velikovsky was (again, in a nod to Freud) convinced that these catastrophes had been repressed as a form of collective amnesia, which explains (conveniently) why most of us who hear about his theories today vigorously reject them as implausible. (Gordin

consciously does not attempt to rebut them, in part because there are no longer any stakes in doing so.)

Under normal conditions, one might expect such a work to pass unnoticed among the other millions of pages of nonsense published in any given year. But, as Gordin chronicles, the conditions surrounding *Worlds in Collision* were just right for a controversy. The book had been released by Macmillan Press, a respected publisher of scientific monographs, which led to an outraged protest by numerous members of the American astronomical community (led in part by Harlow Shapley of Harvard). Their complaint that the book could not possibly

have been peer-reviewed was incorrect—the press had actually subjected it to two separate rounds, and it was tentatively approved even by scientists as interesting and entertaining

although not likely true. But their main objection was that it was being passed off as a work of “science” as opposed to a work of, say, speculative nonfiction. After a series of threats (never organized into a coherent movement) to boycott Macmillan textbooks, the publisher turned the book over to the popular press Double-

day, to the satisfaction of the astronomers. In attempting to draw public attention to the utterly erroneous nature of the book, however, the scientists gave it ample publicity, and it became a best-selling hit.

Gordin takes us through the many phases of the book's history: its origins, its contested publication, its resurgent popularity among antiestablishment college professors and students in the 1970s, and its drop into total obscurity following Velikovsky's death in 1979. This makes for interesting reading in and of itself: The Velikovsky affair is a story of major scientists trying to grapple with what to do about someone they deemed a serious crackpot. Velikovsky, for his part, attempted in fits and starts to find inroads into respectability. Velikovsky was not crazy, Gordin emphasizes. He was simply crankish—totally obsessed, completely convinced, interpersonally difficult. Gordin is extremely sensitive to Velikovsky the human being and makes good use of Velikovsky's expansive personal archives to flesh out the account with key details about his life, methods, and struggles.

The biggest question is, of course, why the scientists raised such an outcry in the first place. Velikovsky's book might have entered obscurity much faster had it not been given so much inadvertent publicity. The answer Gordin gives highlights the particular historical context of Velikovsky: It was a moment of high Cold War anxiety. American scientists had learned from watching the Lysenko affair from abroad that crackpots could be dangerous. In Cold War America, increased government involvement in the funding of science was taken by some to mean the possibility of increased government regulation of science. This Cold War context pervades the early sections of the narrative and crops back up in the reembrace of Velikovsky in years of popular ambivalence to technological progress.

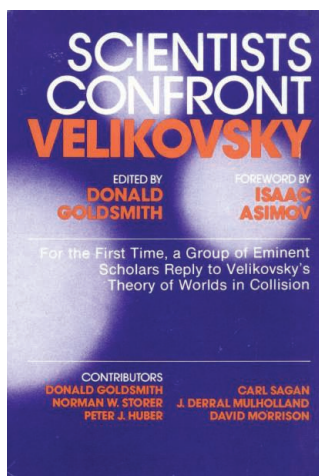
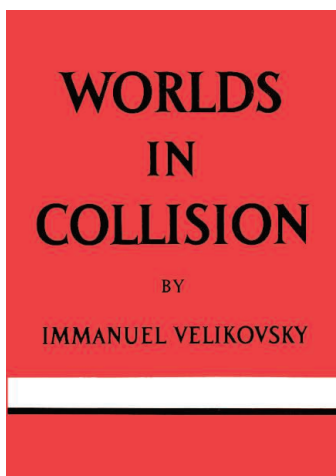
The Velikovsky story also intersects with many other “fringe” communities. Velikovsky's writings and correspondence

The Pseudoscience Wars

Immanuel Velikovsky
and the Birth of the
Modern Fringe

by Michael D. Gordin

University of Chicago Press,
Chicago, 2012. 301 pp. \$29,
£18.50. ISBN 9780226304427.



Clash of views. The covers of *Worlds in Collision* (1950) and a 1970s critique (2).

of historical and present import in the history of Velikovsky's unusual ideas and the efforts of mainstream scientists to explain their erroneous nature to what they perceived to be an unwitting and easily misled public. That such an interesting story could emerge out of what superficially appears to be a very obscure topic is one of the unexpected joys of the book.

The thesis of Velikovsky's major work, *Worlds in Collision* [1950; (1)], sounds so ludicrous that its immense popularity seems incredible: At the time of the events in the

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give Gordin the opportunity to discuss the “rehabilitation” of eugenics, the birth of scientific creationism, and the aforementioned Lysenkoism. In some cases (eugenics in particular), this feels like a bit of a narrative stretch, but it does end up adding breadth to the discussion of pseudoscience in general, and Gordin’s take on each of these topics is original.

Velikovsky cosmic catastrophism is, for Gordin, also a case study on the famously intractable demarcation problem, the difficulty of coming up with firm criteria for what separates science from nonscience, or science from pseudoscience. Along with most philosophers and historians of science, he concludes that the problem is probably impossible to resolve unambiguously: “‘Pseudoscience’ is an empty category, a term of abuse, and there is nothing that necessarily links those dubbed pseudoscientists besides their separate alienation from science at the hands of the establishment.”

This is not to say that Gordin takes an anything-goes approach, that all forms of knowledge are equally valuable. He just doesn’t think there are some magic criteria that will let you sort science from pseudoscience in anything like a purely rational fashion. And indeed, as Gordin notes, the entire meaning of “pseudoscience” is that it mimics “science.” Come up with a criterion—peer review, say—and those eager to prove that they really do science will find ways to implement it as well. (Gordin does, however, hint at a possible strict line between those dubbed “pseudoscientists” and those who are “denialists”—the latter of which he sees as essentially dishonest about their work to cloud consensus on issues affecting monied interests, such as big tobacco or big coal.)

Gordin is careful not to prescribe any pat answers to the question of what to do with pseudoscience. Nonetheless, those who are interested in how bad ideas start, how they diffuse, how they covet and resist confrontation, and how they wax and wane in popularity over time will find much food for thought in this gripping book.

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Supplementary Materials

A symposium at the February 1974 AAAS annual meeting pitted Velikovsky against several critics. Recordings of the talks given there by Velikovsky, J. Derral Mulholland, and Carl Sagan (along with the subsequent question-and-answer exchanges) are available at www.sciencemag.org/content/338/6104/194/suppl/DC1.

10.1126/science.1227959

SOCIAL PSYCHOLOGY

To Endow Trust

Benedikt Herrmann

When the extent of the financial crisis came to light in 2008, former chair of the U.S. Federal Reserve Alan Greenspan had to admit to Congress that he had “made a mistake in presuming that the self interest of organizations ... was such that they were best capable of protecting their own shareholders and the equity in the firms”—a mistake that turned out to be very costly, and not only to the American economy. It might be unfair to blame Greenspan for his misperception of the self-interest of organizations. Until very recently, there was no way for someone to objectively and impartially measure the nature of human social behavior. From Aristotle to George W. Bush, decisions have been made based on personal beliefs about how selfishly or cooperatively other people will act.

However, the situation is changing. Aided by replicable experiments and game-theoretical analysis, intriguing research in a range of disciplines is illuminating the actual nature of human social behavior. Unfortunately, the increasingly specialized language within disciplines makes it difficult for an interested public to follow these advances. Thus it helps to have a lucid and informative account such as Bruce Schneier’s *Liars and Outliers*. The book provides an interesting and entertaining summary of the state of play of research on human social behavior, with a special emphasis on trust and trustworthiness.

Trust forms the fundamental ingredient for the functioning of modern societies and economies. Each day, they involve millions of interactions between strangers. As Schneier nicely demonstrates with many lively examples, our social and economic activities require a high level of trust. If everyone has to be actively monitored to ensure that she or he keeps commitments, our societies could hardly develop.

Schneier (a cryptographer, security specialist, and writer) has a personal interest in

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the issue of how trust and trustworthiness can evolve in societies where people increasingly interact anonymously with one another. The book demonstrates that he has thoroughly surveyed the existing academic literature. Free from preoccupations and personal attachments to any of the scientific disciplines working on the topic, he has compiled a well-structured overview of what research can tell us about how trust and trustworthiness accumulate (although some academic readers may find their publications presented in an unexpected context). This he enlivens by adding real-life experiences on how to build trust and keep trustworthiness alive.

Step by step, Schneier elaborates the evolution of trust from the “atom” of trust, the individual, through to the top level of trust systems, entire societies. At times, the steps are rather large—as, for instance, when he covers in a few pages the whole discussion on the evolutionary history of human social behavior. On other occasions, the steps turn very small, and he may spend many pages explaining specific features of trust and trustworthiness in extensive detail. But in this way, readers become acquainted with doz-

ens of insightful examples of social dilemmas on the levels of the individual, family, firms, and entire societies. In addition, they gain an easy and intuitive introduction to the game-theoretical framework behind much of the academic dispute on the nature of human social behavior.

Some relevant points are, however, missing. With its focus on selfish and social behavior, the book neglects very recent developments in experimental research on the “dark side” of humans—features such as competitive spite and pure aggression. People not only fear falling victim to selfish exploiters of their readiness to behave in a trustworthy manner, they also dread irrational attackers. This raises an additional hurdle to instilling security: Some security technologies adopted to combat overaggression can by themselves damage innocent people, thus increasing distrust. It also would have been nice if Schneier had extended his perspective beyond the United States, as recent research demonstrates that, for instance, norms of cooperation may vary considerably across societies.

Nevertheless, overall *Liars and Outliers* offers a good introduction to human social behavior. Most likely, Greenspan would have enjoyed a read before 2008.

10.1126/science.1224363

Liars and Outliers

Enabling the Trust That Society Needs to Thrive

by Bruce Schneier

Wiley, Indianapolis, IN, 2012.

382 pp. \$24.95, €27.95.

ISBN 9781118143308.

Standards and Infrastructure for Innovation Data Exchange

Data on the global R&D enterprise are inconsistently structured and shared, which hinders understanding and policy.

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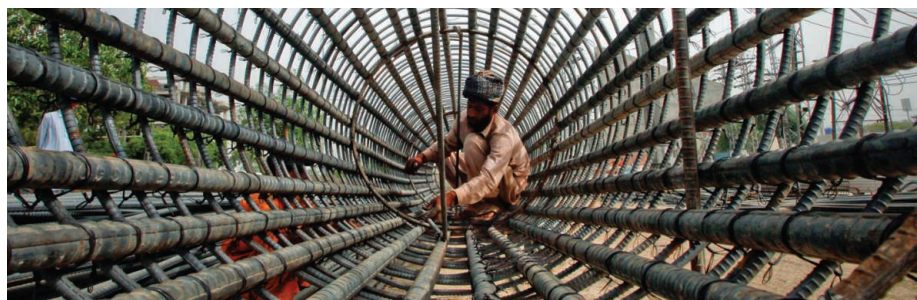
Economic growth relies in part on efficient advancement and application of research and development (R&D) knowledge. This requires access to data about science—in particular, R&D inputs and outputs such as grants, patents, publications, and data sets, to support an understanding of how R&D information is produced and what affects its availability. But there is a cacophony of R&D-related data across countries, disciplines, data providers, and sectors. Burdened with data that are inconsistently specified, researchers and policy-makers have few incentives or mechanisms to share or interlink cleaned data sets. Access to these data is limited by a patchwork of laws, regulations, and practices that are unevenly applied and interpreted (*1*). A Web-based infrastructure for data sharing and analysis could help. Data exchange standards are a first step. We describe administrative and technical demands and opportunities to meet them.

A Distributed Data Infrastructure

There is no single database solution. Data sets are too large, confidentiality issues will limit access, and parties with proprietary components are unlikely to participate in a single-provider solution. Security and licensing require flexible access. Users must be able to attach and integrate new information.

Unified standards for exchanging data could enable a Web-based distributed network, combining local and cloud storage and providing public-access data and tools, private workspace “sandboxes,” and versions of data to support parallel analysis. This infrastructure will likely concentrate existing resources, attract new ones, and maximize benefits from coordination and interoperability while minimizing resource drain and top-down control.

No single organization can or should



manage the infrastructure alone. Governments, nonprofits, and for-profits must collaborate, balancing governance and management, if this vision is to be realized in a way that is sustainable and accountable. This balance would need to minimize user costs while enabling all parties to participate on equal footing. There are successful models in the public and private sectors for aspects of this infrastructure, such as the Common European Research Information Format (CERIF) data-exchange standard applied across several European Union countries. These models need to be expanded to be international and interdisciplinary, which has implications for governance and funding. Their cost-effectiveness needs to be rigorously evaluated.

Major data providers, including federal statistical agencies, standards organizations, and private vendors, as well as user communities, should establish a steering committee. The U.S. National Academies Board on Research Data and Information, and the Committee on Data for Science and Technology of the International Council for Science are among the natural sponsors.

Achieving Broad-Based Participation

The multiplicity of players with different objectives—e.g., multinational corporations, nonprofits, government agencies, academic researchers—constitutes a challenge, but they have potentially complementary roles. What is lacking is coordination in establishing and adopting standards and a platform in which data can be combined.

Government funding agencies provide financial support, can encourage development and implementation of coordinated standards in grant and reporting systems,

and can require that data produced with their support mesh with the infrastructure where possible. For example, the Brazilian Lattes Platform provides an integrated system to manage research information. Lattes is partnering with CASRAI (Consortia Advancing Standards in Research Administration Information), VIVO (an open research-networking community group), and EuroCRIS (Current Research Information Systems) to identify a shared exchange standard. Coordination of funding and standards could drive analysis standards, upon which additional infrastructure can build.

Corporations understand the value of data sharing and have been creating and curating data that support research on research. These include Web of Science citation data, KNODE's work to link papers and patents, and vendors working with universities to collect and manage research data.

Achieving participation requires an understanding of incentives for users. In the United States, the STAR METRICS initiative could transform research if it is part of a wider set of initiatives, but research organizations are concerned about sharing financial information.

Researchers will need to follow standards for reporting activities, but will benefit from improved attribution of their work and interoperability across reporting systems. Citation standards across disciplines and data types—from data sets to algorithms to organisms—need to be defined and implemented. The Institute for Quantitative Social Science has created citation standards (2) that can be used as a starting point for discussions (for example, International Organization for Standardization).

Based on these citation standards, metrics

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should be developed that cover nonpublication research outputs. These could be used in a system to incentivize researchers to participate more broadly in data-sharing activities and allow institutions to track the use and impact of the full array of research outputs. These systems must be carefully considered and tested. The goal is to combine research activity and output data from many sources to support the study and understanding of R&D knowledge flow.

The Role of Open Data Standards

The nonprofit sector is well positioned for defining and maintaining data interoperability standards, because it can bring the many data infrastructure players together with minimal conflicts of interest. Several exchange standards are in use. EuroCRIS has been advancing CERIF, which has been adopted by many government funders and research organizations in Europe. In the United States, VIVO has applied semantic Web storage and retrieval methods to research data. Although national efforts show promise, it is critical to create multidiscipline, multijurisdiction, and technology-neutral standards and vocabularies. Open Researcher and Contributor ID (ORCID) provides a persistent registry for uniquely identifying researchers and is working to automate linkages with research activities. CASRAI provides a peer-reviewed, open dictionary of terminology for the semantics and record-structures of research information.

With these underlying exchange standards, the first step is to create a Web-based registry for data, or expand an existing one such as DataCite, to meet the needs of a global, multidisciplinary, multistakeholder community. Specific Web-based user interfaces (“wrappers”) can interact with the registry and fulfill discipline-specific requirements, such as metadata and related code needs. In addition to biological and physical sciences research activity data, it is important to include organizations like the Social Science Research Network (SSRN), that focus on social sciences and humanities research to ensure the broadest applicability of the infrastructure, reduce future integration efforts, allow for cross-disciplinary data to be combined in innovative research projects, and assist in identifying interdisciplinary work.

Standard technologies could help achieve initial goals. A wrapper protocol like SWORD (Simple Web-Service Offering Repository Deposit) allows users to communicate with a server. SWORD has been successful for publication repositories, and work to extend it to data repositories should be supported. A common set of XML DTD (eXtensible Markup

Language Document Type Definition) tags is needed for consistency and efficiency in content exchanges, simplifying access to data and code stored in various repositories. It is important to provide flexibility, as new technologies will continue to be developed.

A Model for Secure Data Access

Data in this infrastructure should be distinguished in terms of level of sensitivity (3). Nonsensitive data (e.g., aggregated or already in the public domain) can be made publicly available. Privacy laws vary between countries; person-level data has the potential to become sensitive if linked or otherwise enhanced. Processes for managing such data need to be implemented. Protocols for each U.S. project, including any person-level data to be deposited, should receive standard Institutional Review Board approval. Sensitive, individually identifiable and institutional data would be housed in a protected enclave. Our preferred model is a Web-based infrastructure to host public and restricted data, in which sensitive data would be restricted to users who had applied for and obtained access.

A public-private working group should develop recommendations for reconciling existing data privacy laws [e.g., (1)], state and local laws, copyright law, and international standards. This group should discuss laws and regulations needed for providing access while maintaining the security of administrative data gathered by federal agencies. Such a group could be convened by the Confidentiality and Data Access Committee of the U.S. Federal Committee on Statistical Methodology.

The new NSF National Center for Science and Engineering Statistics (NCSES) secure data access facility provides a model of managed access to restricted data, balancing security and access for cleared researchers. Such models can work for commercial providers, such as the tiered security MarketScan repository, which contains medical claims data and is used to support analysis of health-care cost and treatment and patient behavior.

At least three tools would enhance the infrastructure. First, an automated honest broker approach (4, 5) would allow researchers to request access to data and to perform integrated analyses on multiple data sets housed by different providers. Second, review of findings derived from de-identified data sets to verify and preserve confidentiality is a time-consuming manual process; automation of this statistical analysis would facilitate the research process. Third, statistical code that harmonizes variable definitions across data sets could be shared among users who have

been granted access to secure data. These tools will take years to develop; investments in development will prove valuable.

Transforming Research

Researchers lament the lack of data sharing (6). By linking data and algorithms to the infrastructure, researchers could—with permissions—access other research projects, encouraging replication and resource utilization. Users would register for access to security-sensitive parts of the infrastructure, use public data and tools free of charge, and pay for access to private work spaces, intellectual property-controlled data sets, or customized analytic tools. Users could post comments on components. Providers would document their data with standard metadata, including data elements, sample frame, access levels, terms of use, and any fees, which could vary according to the amount and nature of use (e.g., scholarly, commercial, or algorithm development), and vary across providers (e.g., academics and government agencies might set prices to cover expenses, with commercial providers setting higher prices for different functionality and tools). Payment could be managed much the way e-readers manage access to applications and content. Such a structure minimizes centralized support and subsidies; allows data to be maintained by providers who can manage access, data updates, and algorithms for data processing; and users can distribute their own tools and algorithms. These objectives are in line with the U.S. government memorandum on data sharing and privacy (7).

The proposed model offers potential benefits from combining and mining the vast data already available. The first step is to coordinate existing data exchange efforts, the foundation on which the entire effort relies.

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Acknowledgments: We thank J. Saul and J. Hammond for helpful discussion. D.K.G. acknowledges support from NIH grant 1R01AG036820. B.A.W. acknowledges support from NSF grant 1064220. The views and opinions of the authors do not state or reflect those of the NSF.

10.1126/science.1221840

Animal Behavior and the Microbiome

Feedbacks between microbiomes and their hosts affect a range of animal behaviors.

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Human bodies house trillions of symbiotic microorganisms. The genes in this human microbiome outnumber human genes by 100 to 1, and their study is providing profound insights into human health. But humans are not the only animals with microbiomes, and microbiomes do not just impact health. Recent research is revealing surprising roles for microbiomes in shaping behaviors across many animal taxa—shedding light on how behaviors from diet to social interactions affect the composition of host-associated microbial communities (1, 2), and how microbes in turn influence host behavior in dramatic ways (2–6).

Our understanding of interactions between host behavior and microbes stems largely from studies of pathogens. Animal social and mating activities have profound effects on pathogen transmission, and many animals use behavioral strategies to avoid or remove pathogens (7). Pathogens can also manipulate host behavior in overt or covert ways. However, given the diversity of microbes in nature, it is important to expand the view of behavior-microbe interactions to include nonpathogens.

For diverse animals, including iguanas, squids, and many insects, behavior plays a central role in the establishment and regulation of microbial associations (see the first figure). For example, the Kudzu bug (*Megacopta cribraria*), an agricultural pest, is born without any symbionts. After birth it acquires a specific symbiont from bacterial capsules left by its mother. If these capsules are removed, the bugs show dramatic wandering behaviors, presumably to search for symbiont capsules left with nearby eggs (8).

Social contact is another mechanism that can mediate the acquisition and exchange of microbial symbionts. Indeed, a bene-

	Animal	Microbial species or consortium	Interaction with behavior	Implication
Behaviors impact microbiomes	 Kudzu bug (<i>Megacopta cribraria</i>)	<i>Ishikawaella capsulata</i>	When born, bugs feed on capsules of symbionts; if no capsules are present, nymphs wander in search of microbes	Behaviors shape symbiont acquisition
	 Green iguana (<i>Iguana iguana</i>)	Gut microbiota	Juvenile iguanas eat soil or feces to tailor the microbiota to their current diet	Animals may adjust the microbiota at different life-history stages
	 Bobtail squid (<i>Euprymna scolopes</i>)	<i>Vibrio fischeri</i>	Squids eject bioluminescent bacteria daily	Suggests animals can actively control their symbiont populations

Behaviors alter microbiomes. In Kudzu bugs (8), green iguanas (15), and bobtail squid (16), host behaviors alter microbial acquisition and maintenance.

fit of social living in many species may be the transmission of beneficial microbes (9). Koch and Schmid-Hempel have shown that in the case of bumble bees (*Bombus terrestris*), either direct contact with nest mates or feeding on feces of nest mates was necessary for establishing the normal gut microbiota. Bees never exposed to feces had an altered gut microbiota and were more susceptible to the parasite *Crithidia bombi* (1).

Social context also shapes establishment of mammalian microbial associations. For example, chimpanzees from the same community have more similar microbial consortia than do chimpanzees from different communities (10).

Yet, despite these and other examples of behavior facilitating microbial colonization, questions remain. To what extent is juvenile behavior driven by the search for beneficial microbes? How frequently does host choice influence the acquisition of microbial partners? And, if there is strong selection for animals to acquire microbes from each other, what role do beneficial microbes play in the evolution of sociality?

Once host-microbe associations are established, microbes can influence host behavior in ways that have far-reaching implications for host ecology and evolution (see the second figure). Sharon *et al.* recently found that fruit flies (*Drosophila melanogaster*) strongly prefer to mate with individuals reared on the same diet on which they were reared. Antibiotic treatment abolished the mating preference, and inoculation of treated flies with microbes from the dietary media restored the preference, indicating that microbes, and not diet, altered mate choice. Changes in presence of one bacterium, *Lactobacillus plantarum*, were linked to the induction of mating preferences (2). Flies reared on different diets showed differences in major cuticular hydrocarbons, which are known to influence mating, suggesting that the bacteria alter these crucial chemical signals. Similarly, communities of scent gland-inhabiting odor-producing bacteria vary across hyena clans (3), suggesting that microbes could fundamentally alter social interactions in these animals via effects on their chemical communication.

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Microbial effects on animal chemistry also recently have been linked to changes in predator-prey interactions (11) and feeding behavior (12). Females of the African malaria mosquito, *Anopheles gambiae*, use chemical cues released from human skin to locate hosts. By analyzing skin emanations from 48 subjects, Verhulst *et al.* (12)

marine tubeworm *Hydroides elegans*. Bacterial biofilms play a key role in the settlement behavior of many marine invertebrates, from corals to sea urchins. To study the *H. elegans* system, the authors used transposon mutagenesis to knock out a number of genes from the bacterium *Pseudoalteromonas luteoviolacea*, which is required

can be passed between a host and microbe (5, 6). Mice fed with the probiotic *Lactobacillus rhamnosus* fared better in a forced swim test, an indication of lower anxiety, and showed higher expression of γ -aminobutyric acid receptors in the brain. Partial removal of the vagus, a central communication nerve between gut and brain, obliterated the probiotic effect, suggesting that this nerve transmits information on gut bacteria to the brain (5). If beneficial microbes are also found to modify neural and endocrine activity in the brain in other animals, then they have enormous potential to influence how animals behave toward one another.

Experimental approaches that evaluate the behavioral consequences of microbiome manipulation will be key to addressing outstanding questions. Similarly, studies that manipulate animal behavior—for example, by swapping social or mating partners or by enhancing or blocking neuroendocrine function—can be used to identify behaviors that alter microbiomes. Through either approach, the interface between the fields of microbiology and behavior is poised to expand our understanding of complex microbial communities already known to shape animal nutrition and health (13, 14) and to unveil a hidden dimension of animal behavior.

	Animal	Microbial species or consortium	Interaction with behavior	Implication
Microbiomes impact behaviors	 Fruit fly (<i>Drosophila melanogaster</i>)	Gut microbiota	Diet-specific microbiota influence mating preferences	Microbes could drive speciation
	 Mosquito (<i>Anopheles gambiae</i>)	Human skin microbiota	Skin microbes of humans influence attraction to mosquitoes	Differential attraction could impact disease spread
	 Mouse (<i>Mus musculus</i>)	<i>Lactobacillus rhamnosus</i>	The probiotic <i>L. rhamnosus</i> decreases anxiety in mice	Suggests bacteria can alter mood

Microbiomes alter behaviors. In fruit flies (2), mosquitoes (12), and mice (5, 6), microbes alter mating, feeding, and anxiety levels.

found that humans with higher microbial diversity on their skin were less attractive to these mosquitoes. High abundances of *Pseudomonas* spp. and *Variovorax* spp. were also associated with poor attractiveness to *A. gambiae*. These bacteria may produce chemicals that repel mosquitoes or mask attractive volatiles emanating from human skin. Given the importance of chemical communication throughout the animal kingdom, symbiont alteration of host chemistry may be a potent force that shapes many fundamental animal behaviors.

Animal microbiomes often consist of thousands of species of bacteria, many of which cannot be cultivated outside the host. Rapid advances in metagenomics are allowing characterization of microbiomes beyond the few cultivable microbes (10, 13, 14). However, determining which animal behaviors influence and are influenced by microbial symbionts, and the mechanisms underlying these interactions, will require a combination of molecular and experimental approaches. For example, Huang *et al.* have studied the settlement behavior in the

for larval settlement. Mutagenesis of four genes related to cell adhesion and secretion generated bacterial strains that altered worm settlement behavior and metamorphosis (4). It remains to be shown whether similar bacterial phenotypes drive this important life-history transition across metazoans.

Some animal behaviors will be linked to single microbial species, but many will involve communities of multiple microbial species. It is unclear how fluctuations in the microbiome throughout the host life cycle drive behavioral traits and vice versa. Another challenge is to identify when behavior shapes the microbiome, when the microbiome shapes behavior, and when there is a complex feedback between the two. This requires manipulative experiments and will be facilitated by studying the underlying mechanisms by which signals are sent between hosts and microbes.

Recent experiments with mice, showing that the gut microbiome can influence stress, anxiety, and depression-related behavior via effects on the host's neuroendocrine system, provide insight into how information

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Acknowledgments: This perspective was made possible thanks to NSF meeting grant IOS 1229439 "Meeting: The Future of Research in Animal Behavior." We thank B. Parker, A. Laughton, B. Wehrle, C. Fontaine, and D. Ditmarsch for discussion and comments.

10.1126/science.1227412

CELL SIGNALING

Putting the Squeeze on Phototransduction

Emily R. Liman

The study of phototransduction—the conversion of light into electrical signals—in the fruit fly *Drosophila melanogaster* has presaged many advances in our understanding of cell signaling and sensory biology over the past two decades. Notably, it was in this system that the transient receptor potential (TRP) ion channels were identified (1–3), a family that numbers in the dozens in vertebrate genomes (4). Vertebrate TRP channels, which were first reported as the transducers of sensory processes including thermosensation and pheromone detection (5, 6), have since been found to partici-

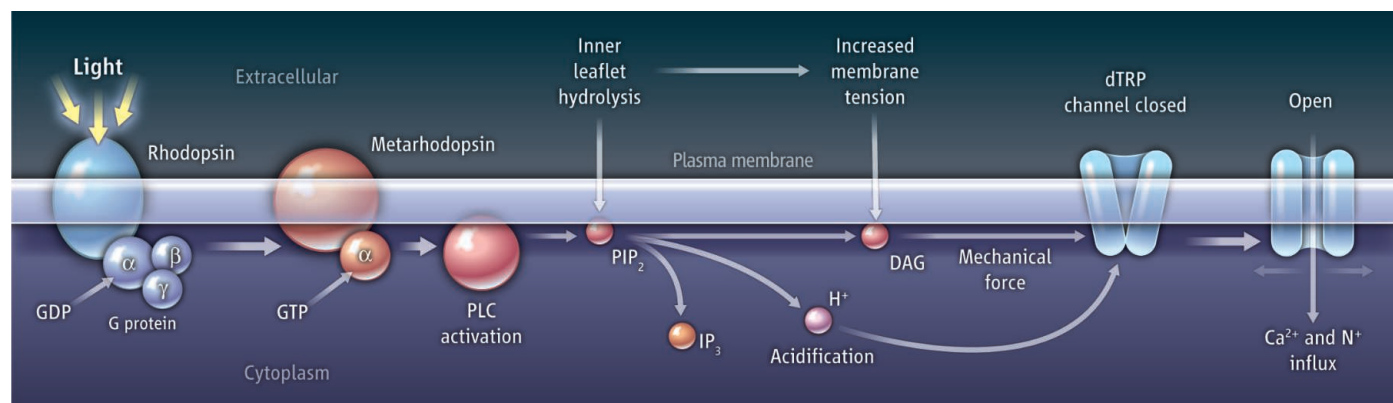
culminate with the opening of two ion channels, the transient receptor potential (dTRP) and its homolog TRP-like (dTRPL). PLC hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP_2) into inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 does not mediate phototransduction (9), and a direct role for DAG in phototransduction (10) remains speculative. Thus, the search for the messenger that can link PLC activity to gating of TRP channels goes on.

Besides generating IP_3 and DAG, activation of PLC has two other direct consequences: depletion of PIP_2 and the genera-

Changes in the physical properties of the lipid bilayer stimulate the opening of ion channels during fly phototransduction.

tion from the membrane. The conclusion follows from a striking observation that light causes a contraction in the fly photoreceptors. Hardie and Franze quantitate this movement with atomic force microscopy and show that it reaches a peak of ~400 nm, with an onset that precedes the onset of the phototransduction current. This contraction depends on the enzymatic activity of PLC but not on dTRP channel activity. Hence, it occurs in the right place within the transduction cascade to contribute to TRP channel gating.

What generates movement of the rhabdomere? Hardie and Franze argue that it



pate in nearly all areas of cellular physiology (4). For fly phototransduction, one question has remained conspicuously unanswered: How does a light stimulus lead to the opening of TRP channels? On page 260 of this issue, Hardie and Franze (7) report a surprising link between light-activated changes in membrane tension and the opening of TRP channels that may be the long-awaited answer.

Phototransduction occurs in eight photoreceptor cells that make up an ommatidium, the unit of the fly's compound eye (8). The photoreceptor cell projects 30,000 microvilli that form a stack called the rhabdomere, in which phototransduction occurs (4). Phototransduction in the fly (see the figure) is initiated upon photoisomerization of the light-sensitive pigment rhodopsin, which then sequentially activates a heterotrimeric G protein and the enzyme phospholipase C (PLC). The cascade

tion of a proton, the latter of which is often overlooked. Depletion of PIP_2 can affect the activity of ion channels and transporters, including TRP channels (11). This has generally been ascribed to specific interaction between the channel and the phospholipid, as visualized in the structure of the inward rectifier K^+ channel (12). However, there is little evidence of a direct interaction between *Drosophila* dTRP channels and PIP_2 (8). Instead, attention recently focused on a possible role for the proton when it was reported that light triggers a PLC-dependent acidification of the fly rhabdomere (13). Acidification activates dTRP and dTRPL, but only when PIP_2 has been depleted. This suggests that it is the coincident depletion of PIP_2 and increase in the local concentration of H^+ that activates dTRP channels during fly phototransduction.

Curiously, Hardie and Franze now report that it is not PIP_2 depletion per se that activates the TRP channels, but rather the change in membrane tension as a result of PIP_2 deple-

Fly phototransduction. Light stimulation of rhodopsin in a fly photoreceptor cell leads to the hydrolysis of PIP_2 by phospholipase C (PLC), generating IP_3 , DAG, and a proton. Conversion of PIP_2 to the slimmer molecule DAG on the inner leaflet of the membrane increases membrane tension, which opens dTRP channels and causes contraction of the microvilli. Intracellular acidification also activates the dTRP channels.

reflects a response to the change in membrane tension that occurs when PIP_2 , which contains a bulky head group, is converted to the slimmer DAG molecule in the inner leaflet of the plasma membrane. To alleviate this stress, the inner leaflet compresses, causing a tiny constriction in the diameter of the microvilli. Because microvilli are arranged in stacks of ~1500, a small change in the diameter of the microvilli is amplified into a large change in length of the rhabdomere. This result is remarkable because, although it is well recognized that changes in phospholipid composition can change

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membrane curvature, bilayer thickness, and membrane stress (14), there was little prior evidence that PIP₂ hydrolysis could produce a measurable change in the shape of a living cell.

Hardie and Franze substantiate their hypothesis by showing that light can activate a well-studied mechanically gated ion channel, gramicidin, when inserted in the photoreceptor plasma membrane (14). To show that membrane tension gates dTRP channels, the authors exposed photoreceptors to hypo-osmotic solutions expected to have the same effect as PIP₂ hydrolysis in reducing crowding in the membrane. Indeed, hypo-osmotic solutions, though not sufficient to activate the dTRP channels, potentiated the photoreponse. Finally, to independently manipulate membrane tension, Hardie and Franze used cationic amphipaths, which are expected to counteract the effect of PIP₂ depletion by preferentially increasing crowding in the inner leaflet of the lipid bilayer. Consistent with the interpretation that the generation of membrane tension is required for phototransduction, several unrelated cationic amphipaths reduced the electrical response of the photoreceptor to light.

So is the mystery solved? The study of Hardie and Franze and others (15) support the intriguing possibility that changes in the physical properties of the lipid bilayer constitute a direct stimulus that opens TRP channels during phototransduction. This is not so surprising given the structural evidence that that conformational states of some channels can be differentially stabilized by nonspecific interactions with the membrane bilayer and that, consequently, changes in membrane tension can gate channels not previously thought to have any role in mechanosensing (16). What is surprising is that the change in lipid composition after PLC activation can create a change in membrane properties sufficient to change the diameter of microvilli and the shape of the rhabdomere. This hypothesis is naturally overly simplistic, overlooking, for example, the protein scaffolds that contribute to the formation and maintenance of the microvilli, which could underlie the observed movement of the rhabdomere. Also, granted that the microvilli contract as proposed, it is not clear whether this movement gates the channels or represents a parallel response to the change in membrane properties, relieving membrane tension and opposing channel activation.

Can changes in membrane properties sufficient to gate TRP (or other) channels be generated in other cell types? Do they only arise in small confined processes, where PIP₂

can be rapidly depleted and tension is not easily dissipated? Such confined processes might include dendritic spines in neurons, cilia, filopodia, and microvilli—places where mammalian TRP channels are enriched (7). The study of Hardie and Franze highlights the emerging concept that transmembrane proteins are sensitive to the membrane environment (16)—an environment that is dynamically regulated by extracellular and intracellular signals.

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10.1126/science.1229909

DEVELOPMENTAL BIOLOGY

Physical Biology Returns to Morphogenesis

Ray Keller

Understanding how cells coordinately shape an organism is grounded in a history of biophysical analyses of the molecules, mechanics, and forces that govern cell movements.

One of the enduring mysteries of developmental and cell biology is morphogenesis, the process of how the heritable body plan, in both its general aspects and in the detailed features of the adult, is shaped at every stage from the fertilized egg. Physical analysis played a large role early in the history of its study, then was largely ignored for a time as other approaches held sway, but has recently regained a central role as attention has turned anew to solving the riddle of how tens, hundreds, or thousands of embryonic cells act in coordinated fashion in so-called “mass” or “collective” cell movements, to shape the body plan of multicellular animals.

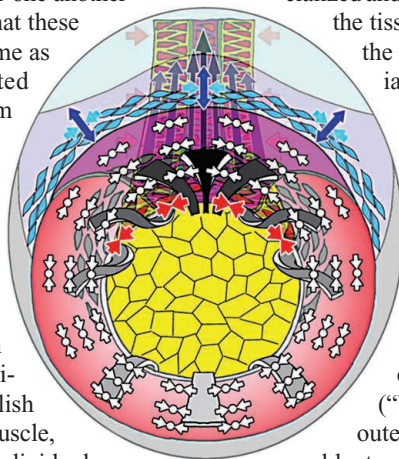
The early embryologists of the *Entwicklungsmechanik* (developmental mechanics) movement, which spanned from the mid-19th to the mid-20th century, analyzed morphogenesis (“shape generation”) as a physical, mechanical problem of how forces generated by cells reshape the embryo. These included forces produced by oriented cell division and growth, directed cell crawling, and bending of cell sheets by the integration of local cell shape changes (1). Amoeboid movement of

cells had been described, but the problem of how local behaviors and forces are integrated to produce the smoothly coordinated movements of thousands of cells, as revealed in Walter Vogt’s dye maps of the amphibian embryo, seemed to many a question beyond answer by reductionist approaches (2).

However, Johannes Holtfreter, a bold and perhaps the most creative embryologist of the day, was not intimidated. He pioneered two concepts that offered physical mechanisms to account for the global “mass” movements of embryonic tissues. These concepts inspired two schools of investigation that have defined the field of morphogenesis and raised biophysical questions that today lie at the heart of the problem of integrated cell movements. The first was the concept of cell “recognition” and “selective affinity” of cells and tissues (3, 4), and the second was the concept of mechanically integrated cell motile behavior (1, 5, 6). Both had been offered in one form or another before, but Holtfreter articulated them and gave them experimental life as no one else had. He developed a physiological saline (“Holtfreter” solution) that supported the culture of amphibian embryonic cells and tissues. This allowed him to dissect the embryo, study the cell and tissue arrangements in situ, and observe the behavior of tis-

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sues and cells as individuals, groups, or large aggregates of different types of cells, *in vitro*. He combined tissues of the three primary germ layers—ectoderm, mesoderm, and endoderm—and found that they recognized one another as alike or different (cell recognition), that they could have either a positive or a negative “affinity” for one another (selective affinity), and that these affinities changed over time as developmentally regulated processes (3). Endoderm and ectoderm develop a negative affinity and isolate from one another, whereas mesoderm retains a positive affinity for both, reflecting their behavior during gastrulation when the three germ layers move into a definitive arrangement to establish the skin, gut, skeleton, muscle, and connective tissue. Individual cells, dissociated and reaggregated as mixed populations, likewise showed selective affinity and ordered themselves just as the tissues had in earlier experiments (4). Malcolm Steinberg (7, 8) found that different types of chicken embryonic cells would likewise sort into homotypic (like with like) arrays and then arrange into segregated tissues. Instead of the broader concept of “affinity” used by Holtfreter, Steinberg made a thermodynamic argument in the differential adhesion hypothesis (DAH) that the path and final order of tissue arrangements are driven by the “work of adhesion” between heterotypic and homotypic cells. As they sample their adhesive environment, cells exchange weaker adhesions for stronger ones, maximizing the area of the strongest adhesions, and thereby minimizing the interfacial free energy of the system. Tissues behave as immiscible liquids, with an adhesion-driven surface tension. As motility allows cells to sample their adhesive environment, they flow into new patterns of association (adhesion-guided multicellular assembly). The DAH had great appeal from the 1960s through the 1980s because of its simple mechanism of causality from gene to embryonic form: Genes encode cell-surface adhesion molecules; mechanically competitive differential adhesion occurs; and cells and tissues sort and flow to yield a properly shaped embryo. Cell motility was essential but unspecialized, and sufficient to “stir the pot,” allowing cells to sample hetero- and homotypic adhesions and then sort. The DAH supported a “compositional” notion of mechanism in which identifying a list of components was



Forced to move. The diagram illustrated the major movements of convergent thickening (white arrows) and convergent extension (red, blue, and black arrows) in frog gastrulation.

key, and it left the central, experimental paradigm of that era—identify genes, “knock” them out, and see what stops—as the centerpiece of developmental biology.

But Holtfreter was also a principal founder of the “cell behavior” school, which held that cell behavior is not generic but specialized and mechanically integrated at the tissue level. He observed that the outer cells of the amphibian embryo were mechanically linked into a sheet and that a subset of these cells became bottle-shaped (“bottle cells”), bending the outer layer inward to form the blastopore. Holtfreter cultured these cells in clusters and observed protrusive activity—extensions of their margins that attach to and exert traction on the substratum—and directional crawling. He postulated that in the embryo, these cells migrate directionally inward, and because of their mechanical linkage to the rest of the outer layer of the embryo, they “tow” it into the embryo, forming the precursor to the gut (1, 5). Holtfreter described other cell shape changes, modes of cell crawling, and cell-cell contact behaviors, and proposed mechanisms by which the force-producing potential of these behaviors could drive morphogenesis of tissues. His work was extended by time-lapse movie studies of the echinoderm embryo by Trigve Gustafson and Lewis Wolpert (9), among others. Collectively, this work established the concept that cells do more than randomly move to sample adhesive possibilities, and instead have polarity, directionality, mechanical linkages to one another, mechanical integration of behavior, and forces.

But there was a long road ahead to establish the study of cell behavior and cell sociobiology as legitimate disciplines, and further yet to forge a coherent mechanism of integrating local behavior into global forces. The journey was advanced greatly by Michael Abercrombie and John Philip Trinkaus. Abercrombie and his associates defined and quantified major features of cell motility and contact-mediated cell behavior in culture. They demonstrated the spreading of cell populations by density-dependent contact inhibition of movement, the contact-induced retraction of cell protrusions, and the tendency of cells

to turn (10). Strongly influenced by Abercrombie, Trinkaus and his associates applied lessons learned *in vitro* to morphogenesis in embryos, including avian neural crest cell migration, echinoderm gut invagination and mesenchyme cell migration, and cell motility and epiboly in the teleost fish. Trinkaus thought of specific embryonic cell motilities in terms of how they generate forces that shape the embryo (11). These concepts of behavior developed in parallel with many studies on the mechanobiology of the internal cytoskeleton (actin-containing microfilaments, microtubules, and their respective molecular motors, myosin and kinesin, and dynein), and the discovery of cell surface receptors with both mechanical (adhesive) and sensory (signaling) properties, such as cadherins (cell-cell adhesion molecules) and integrins (cell-matrix adhesion molecules), among others. Together, the cell behaviorists and cell biologists provided substance to the idea of cells as small force-generating machines with regulated, specialized behaviors that exert force on other cells and on the extracellular matrix. The question of how these local forces are integrated to deform the whole was addressed in a pioneering study of nervous system morphogenesis. Antone Jacobson and Richard Gordon (12) combined microsurgical manipulations, time lapse movies of tissue deformation, and computer simulations to show that the dramatic convergence (narrowing) and extension (lengthening) of the neural plate of the amphibian, prior to its rolling into a tube, could only be explained by the summed forces of endogenous, locally autonomous cell shape changes, and external lengthening forces from the attached midline notochord/notoplate tissue (see the figure). Other notable combinations of time-lapse imaging, experimental manipulation, and computational modeling followed and highlighted the potential of combining engineering and mathematical approaches with dynamic descriptions and experimental manipulations (13, 14).

Despite these advances, the question of how local cell behavior and forces are physiologically and mechanically integrated over hundreds or even thousands of cells remains a challenge, to be met with new technologies and biophysical approaches to explore possible answers. The development of vital fluorescent dyes, high-sensitivity cameras, videomicroscopy, digital image processing, and the laser scanning confocal microscope have allowed high-resolution imaging and measuring parameters of cell movements in dense populations of living cells in embryos. The development of green fluorescent protein

(GFP) was a key advance, as it can be genetically regulated to target specific cell populations. Chimeric proteins of GFP and cellular components can be made to follow the nuts and bolts of the cell's morphogenic machinery—molecular constituents of the cytoskeleton, the cell membrane, adhesion molecules, and signaling molecules. Small-molecule inhibitors allow acute perturbation of molecular function, and transgenic embryos allow expression of negatively and positively acting proteins in a specific time and tissue context; together, these tools are a powerful means for determining how the same or similar molecular constituents are used repeatedly in different ways during morphogenesis. The forces and mechanical properties of tissues, cells, and molecular assemblies can be measured with a refined use of old methods and also new ones, including bending of computer-controlled glass fibers to measure tissue mechanical properties and forces; aspiration of surfaces with suction; atomic force microscopy to

measure mechanical properties of cells (elasticity, viscosity); traction force microscopy (deformation of gels or microscopic pillars) to measure cell traction on substrates; optical traps and magnetic beads to measure molecular-level mechanical properties; and laser ablation of cellular and cytoskeletal components. Most important, applying the principles of engineering and soft-matter physics to cell and tissue morphogenesis allows the construction and testing of hypotheses that are meaningful at embryonic length scales, rather than the often deceiving extrapolation from mechanics on the length scale of our experience (15). Recent approaches combining, for example, computational modeling, biomechanical measurement, and experimental manipulation have advanced our understanding of how local cell behavior and mechanical interaction of tissues drives morphogenesis (16–18). Physical biology has indeed returned to a central and essential role in analysis of integrated cell movements.

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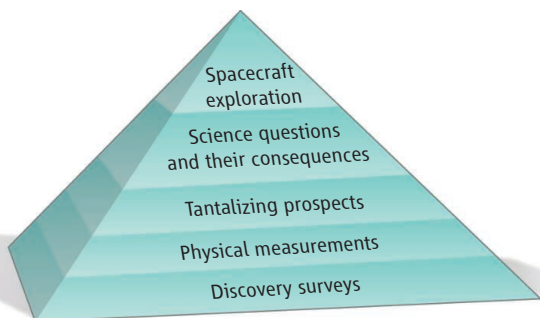
PLANETARY SCIENCE

A Golden Spike for Planetary Science

Richard P. Binzel

Vesta is the second most massive asteroid in our solar system and provides the opportunity to study the early Earth as a developing planet while it was still barely one-sixth the size of the Moon. Add to that what appear to be samples from this once molten protoplanet and you have the decades of case building and motivation for the spacecraft exploration of Vesta—the Dawn mission (1). Results of this mission, reported by Prettyman *et al.* (2) on page 242 and Denevi *et al.* (3) on page 246 in this issue, not only tell us about the birth and evolution of terrestrial planets, but also offer new insights into the distribution of water in our solar system.

Prettyman *et al.* link conclusively the elemental chemistry of Vesta to the howardite, eucrite, and diogenite (HED) classes of basaltic meteorites and their detailed record for the earliest phases of planetary evolution. Although this connection has been long suspected (4, 5), Vesta now solidifies its place



Pyramid building. Planetary exploration begins with discovery, often in the course of surveys. Physical measurements, with increasing precision, bring to focus particularly tantalizing objects. Science questions become refined through both observation and theory, often to their limit. Spacecraft exploration capable of delivering consequential breakthroughs forms the capstone.

as the fourth planetary body from which we have definitive samples in our laboratories [Earth, Moon, and Mars constitute the first three; Japan's successful asteroid sample return (6) adds one more]. As often happens in planetary exploration, confirming the expected also uncovers the unforeseen: Prettyman *et al.* find an abundance of hydrogen within the oldest regions of Vesta. Supporting the logical interpretation that the detected H is bound as H₂O, volatile degassing of water is a natural explanation for pit-

The progression from astronomical observation to geochemical analysis epitomizes advancements in planetary exploration.

ted geologic structures examined by Denevi *et al.*

As interesting as Dawn's particular findings for Vesta are, lost in the details is the perspective of embracing how much of what we already knew about Vesta was confirmed by the in situ measurements. No mission wants to be viewed as having done incremental science, but when that progression hews both a capstone and a deeper scientific context, the value is undiminished. It is inherent that planetary exploration must advance itself literally from the ground up, historically beginning with Earth-based telescopes and now increasingly from space. Surveys to find planets (within our solar system or beyond), dwarf planets, Kuiper belt objects, comets, and asteroids, or to reveal particles and fields, present space agencies with numerous destinations, vastly eclipsing available resources. Such breadth of possibilities is like the base of a pyramid (see the figure). Physical investigations to discern basic properties (for example, mass,

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spin, size, shape, configuration, density, and composition) logically lead up to the next level. Invariably, some objects attain a still higher level by being especially remarkable, inexplicable, quintessential, or tantalizing. Prioritizing which objects to study is a difficult task, but the fundamental depth of the scientific questions that an object raises and the consequences of the answers usually prevail among all missions within feasible technology and cost.

Vesta was recorded at the base of the pyramid with its 1807 discovery as the fourth asteroid known, fostering the modern view that the “missing planet” between Mars and Jupiter is one that never completely coalesced. Physical observations first reported in 1929 (7) were astonishingly correct in revealing that Vesta has a variegated surface. Modern Vesta science began in the 1970s with the finding that its surface colors matched those of the HED meteorites (4). As the only distinctive asteroid-meteorite match among all large asteroids (5), Vesta raised consternation over how samples from the asteroid belt might ever reach Earth (8). Controversy continued until the finding of a Vesta-like family of asteroid debris in heliocentric orbits emanating from Vesta (9) and Hubble images (10) that revealed the “smoking gun” of a huge impact basin (named Rheasilvia) as their source. These Vesta chips, leaving a trail all the way to where Jovian resonances could deliver them to Earth (11, 12), completed the pathway.

With the confidence that we have pieces of Vesta in our laboratories, increasingly detailed maps (13, 14) showed distribu-

tions of pyroxene-rich surface basalts, more deeply excavated plutonic rocks near the basin, and widespread mixtures of the two. These three components fit exactly to the laboratory mineralogy for HED meteorites. Their measured ages, several hundred million years older than lunar basalts (15), branded Vesta as the most ancient terrestrial world (16). With Vesta so enticingly unveiled and scientists at the ready, the Dawn mission was born.

Dawn’s crowning success at Vesta was the delivery of instruments that require proximity, exemplified by the gamma-ray and neutron measurements of Prettyman *et al.* yielding elemental abundances that unequivocally confirm the meteorite links. While pyroxene mineral mapping from orbit (17) contends as clinching that link, these maps instead provide geologic context to the mineralogy match known for decades (4, 13). The cacheing of volatiles at Vesta reported by Denevi *et al.* accentuates the pervasiveness of water throughout the solar system. Yet, Dawn fell short of fulfilling the apex of planetary exploration’s pyramid through decisions forced by budgetary constraints and cost overruns. Geophysics was sacrificed at Vesta’s altar as two instruments with transformative prospects, a magnetometer and laser topography mapper, were removed to avert Dawn’s cancellation. Key planetary questions on early dynamos and protoplanet core-mantle-crust differentiated structure, addressable only in situ at Vesta, remain preserved there.

Finding Vesta not to be as predicted, a terrestrial-like protoplanet (1, 16), would

have been a complete surprise. Happily, the geologic context resolved for this terrestrial world now confirmed as predating the Moon will intrigue scientists for decades. Dawn’s direct affirmation that a complex formation story and meteorite link could be determined correctly from Earth, roughly 200 million km away, is a triumph for how the pyramid of planetary exploration is built. The underpinning foundations that can be mortared together only by a spacecraft’s confirming ground truth data, in turn, support other inferences for planetary places unlikely to be visited in the near future.

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Published online 20 September 2012;
10.1126/science.1228328

BIOGEOCHEMISTRY

Downsizing the Deep Biosphere

Kai-Uwe Hinrichs¹ and Fumio Inagaki²

The seabed of the ocean harbors diverse microbial communities that are adapted to a wide range of chemical, physical, and geological conditions. These communities may extend several kilometers below the seafloor (1–4), and since the 1990s, ocean scientists have assumed that the biomass of this deep bio-

sphere dwarfs that in the overlying ocean (1, 5). When expressed as mass units of carbon, between 10 and 30% of Earth’s life is estimated to be buried as microbial biomass beneath the seabed (1, 5, 6). However, a recent study by Kallmeyer *et al.* (7) suggests that this fraction may be only 1%.

How did these researchers arrive at these estimates, and what is their importance? Concentrations of microbial cells are commonly determined via direct-counting techniques (1, 7); alternatively, chemical approaches targeting lipids as biomass proxies have been used (6). All existing global

A recent study provides new constraints on the size and distribution of microbial biomass beneath the ocean floor, but key factors remain uncertain.

estimates of seafloor biomass are based on the determination of environmental gradients of cell or lipid concentrations, estimation of the global volume of habitable sediments, some level of habitat differentiation (e.g., open ocean versus continental margin), and conversion of the measured variables into biomass (see the figure). Numerical functions are used to extrapolate the observed concentrations into unknown territories, both vertically and laterally, and yield predictions of concentration within sediments considered habitable, which are then added up to a global figure of cells or lipids

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sphere dwarfs that in the overlying ocean (1, 5). When expressed as mass units of carbon, between 10 and 30% of Earth’s life is estimated to be buried as microbial biomass beneath the seabed (1, 5, 6). However, a recent study by Kallmeyer *et al.* (7) suggests that this fraction may be only 1%.

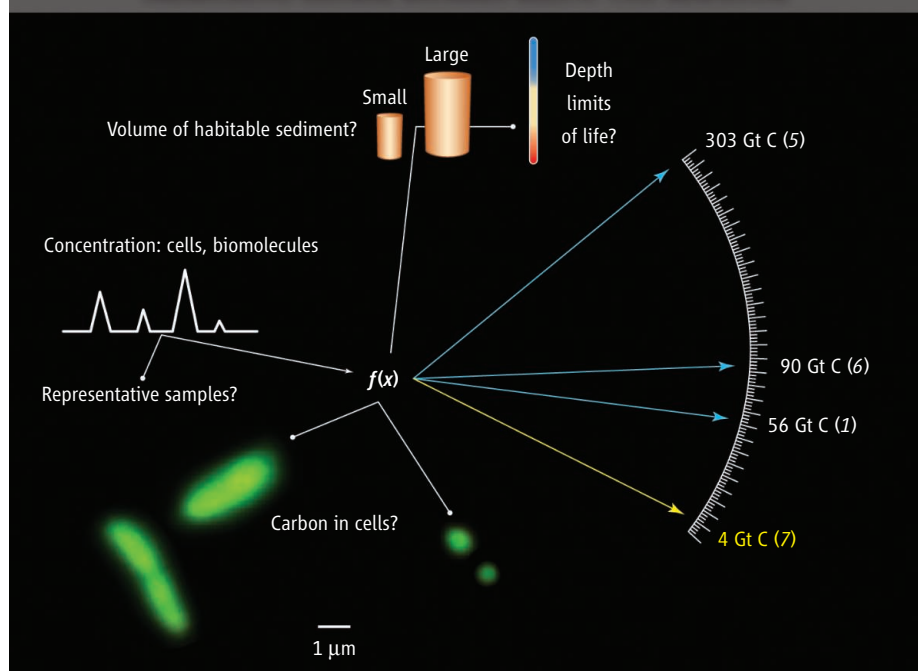
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MEASURING GLOBAL BIOMASS BELOW THE SEAFLOOR



From cellular to global scales. Estimates of the size of Earth's marine deep biosphere—below the seafloor—are based on quantification of cells or biomolecules in deep sediment cores. Extrapolation from sediment cores to global scales requires careful selection of representative environments and informed assumptions concerning both the vertical extension of habitable zones and the body mass index of buried microbes. Estimates of subsurface biomass are in billions of tons of carbon (Gt C). The new estimate by Kallmeyer *et al.* (7) is much lower than previous ones. In images at the lower left, the large cells are *Escherichia coli* and the small cells are indigenous subsurface cells.

buried in the seabed. To place these global inventories in a context of other carbon reservoirs, the last critical step involves conversion of cells or lipids into mass units of cellular carbon.

The new study (7) improves several of the aforementioned steps. First, carefully selected sites have been added to the existing global data compilation, which was biased toward continental margin regions. The new data set includes important end-member sediment regimes, such as large areas of the open ocean that harbor very low cell concentrations. The study used a recently devised cell separation technique for counting these sparse microbial populations (8), which resulted in much improved data quality for such low-biomass terrains. Although cell numbers in much of the open ocean have little influence on global inventories, the ability to quantify cells in these poorly explored sediments provides new clues regarding the factors that control cellular abundance. The authors demonstrate that sedimentation rate and distance to land are reliable predictors of cell abundance; their model incorporates this observation and extrapolates cellular abundance to these vast unsampled territories. The result is a more nuanced representation

of the diverse habitats found underneath the ocean.

Lipp *et al.* (6) had suggested that the amount of organic matter serves as a predictor of microbial biomass in marine sediments. They used microbial lipids as biomass proxies and showed that these correlate with organic matter content. Such a relationship is consistent with biomass being linked to sedimentation rate and distance to land, because rapidly sedimenting continental margins contain more organic matter than low-sedimentation areas in the open ocean. Lipp *et al.* (6) and Kallmeyer *et al.* (7) used similar approaches to determine the volume of habitable sediment, but the former arrived at biomass estimates of around 90 billion tons of carbon. The discrepancy can partly be attributed to the underrepresentation of open-ocean sites in the Lipp *et al.* study, but may additionally be caused by fossil portions of biomolecules that are preserved in sediments (9, 10). Chemical approaches targeting biomass will likely benefit from further refinements.

The last critical step in studies based on cell counts is the choice of the cell-to-carbon conversion factor—not an easy task, given the large range of size-dependent cellular

carbon contents (see the figure) and the difficulty of directly measuring this property for indigenous microbes. A factor of 6 difference in biomass estimates can be attributed to the use of different conversion factors chosen in past studies. Kallmeyer *et al.* (7) use the lowest value to date, but they back up their choice convincingly with statistical data on the size distribution of indigenous cells.

Although the new study constrains some of the environmental factors that control the population density of subsurface microbes, critical gaps remain before we can confidently model the size and extension of deep subsurface life. For example, the limits of life that define the habitable zones remain poorly constrained. Is the assumption that temperature sets its lower boundary justified, as assumed in all studies quantifying subsurface biomass, or are other factors such as pore space (11) or energy availability (12) important, too?

The arguments that the biomass pool below the seafloor may be “only” as large as the pool above are compelling. Nevertheless, this insight about lower biomass says little about the importance of this ecosystem, which bridges the biological and geological element cycles. Microbial life at this interface directly influences whether several important elements are sequestered for millions of years or returned to the ocean as active agents with an impact on life and climate. Only a better understanding of microbial activity and physiology, from the cellular to the system level, will enable informed conclusions regarding the deep biosphere's impact on global biogeochemical cycles.

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10.1126/science.1229296

Cilia Discern Left from Right

Dominic P. Norris and Daniel T. Grimes

Although the human body shows left-right (L-R) mirror symmetry when viewed externally, the placement and patterning of the internal organs and associated vasculature are strikingly asymmetrical. In the mammalian early embryo, L-R symmetry is broken by the action of rotating cilia—small hair-like protrusions on the surface of cells—in a pit-like structure called the node (see the figure). These cilia generate a unidirectional flow of fluid across the node from right to left (1). This nodal flow breaks L-R symmetry by driving asymmetries in gene expression and Ca^{2+} signaling in cells at the periphery of the node. On page 226 of this issue, Yoshiba *et al.* (2) reveal a mechanism by which the mouse embryo senses nodal flow.

Surrounding the node pit with its rotating cilia are crown cells bearing immotile cilia (3). A long-standing model, the “two-cilia” hypothesis, argues that the peripheral immotile cilia sense the flow generated by the centrally located motile cilia (3, 4). The asymmetric signals that are induced by flow are first apparent in the crown cells. Here, the secreted signaling molecule Nodal is expressed more

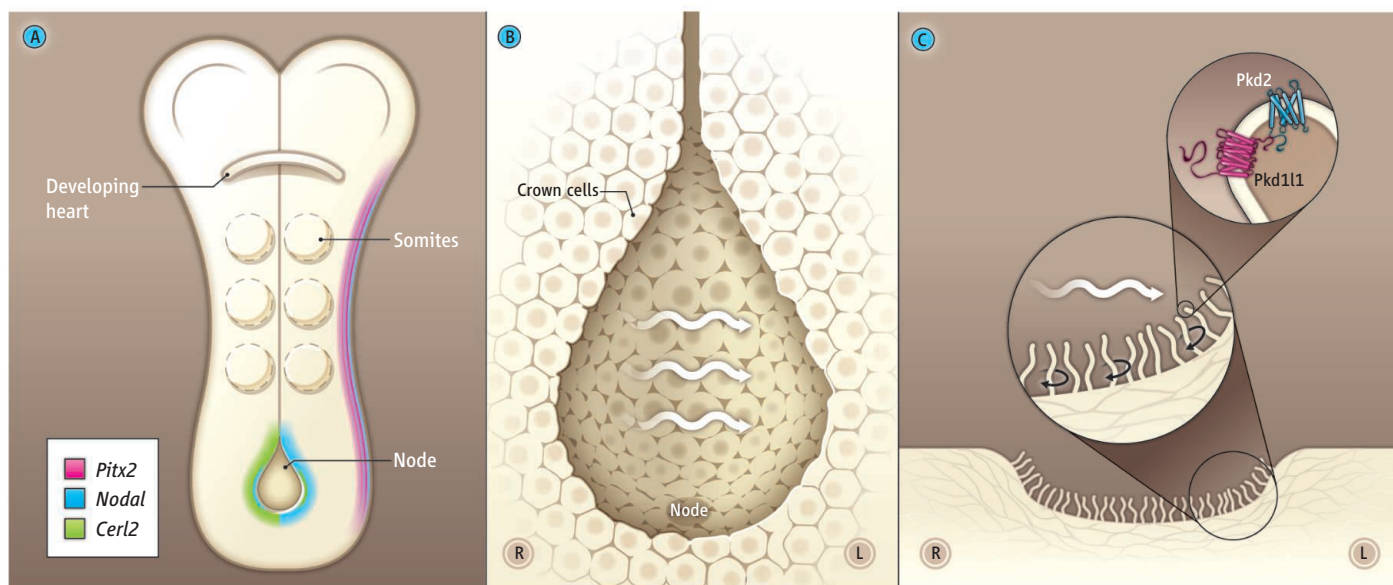
strongly on the left than on the right, whereas its antagonist, the secreted molecule Cerberus-like 2 (*Cerl2*), is expressed with the opposite bias. Subsequently, Nodal and its target, the gene encoding the transcription factor Paired-like homeodomain transcription factor 2 (*Pitx2*), are expressed more laterally along the left but not right side of the embryo. Although asymmetric Nodal is only briefly expressed, the expression of left-sided *Pitx2* is maintained longer and controls asymmetric organ patterning. The link between the asymmetric gene expression in the cells surrounding the node and the flow of fluid across the node from right to left has, however, remained unresolved.

The transmembrane cation channel Polycystin-2 (*Pkd2*) is required for L-R patterning in the vertebrate embryo and is implicated in the asymmetric elevation in intracellular calcium concentration (“ Ca^{2+} spike”) that occurs to the left of the node in response to flow (3, 5). The mouse *Pkd2* gene is ubiquitously expressed, making its site of action uncertain. Yoshiba *et al.* analyzed *Pkd2*-deficient mutant mouse embryos. By rescuing expression of the gene in only specific regions—the entire node (crown and pit cells), crown cells only, or solely pit cells—the authors demonstrate that *Pkd2* expression

Determination of vertebrate left-right body asymmetry requires immotile cilia that sense fluid flow generated by nearby motile cilia.

in node pit cells is not sufficient to rescue L-R development. By contrast, expression of *Pkd2* in just the crown cells rescued normal L-R development, demonstrating that *Pkd2* is specifically required in these peripheral cells, where most immotile cilia are located. *Pkd2* protein normally localizes to cilia in the node (3), suggesting that its role during the breaking of L-R symmetry is played within cilia. The *Pkd2*^{lrm4} mutant mouse (6) exhibits severe L-R defects similar to those of *Pkd2*-deficient mice (5). Yoshiba *et al.* show that although the mutant *Pkd2*^{lrm4} protein retains normal Ca^{2+} channel activity, it fails to localize to cilia within the node, supporting the idea that *Pkd2* normally acts in cilia. Asymmetry of *Cerl2* gene expression, which is stronger on the right than on the left side of the node, depends on *Pkd2* (7); Yoshiba *et al.* go further, demonstrating that *Cerl2* is a target of flow-induced *Pkd2*-mediated signals.

Because *Pkd2* is a Ca^{2+} channel and a Ca^{2+} spike around the node is stronger on the left than on the right (3), it has been thought that *Pkd2*, acting downstream of flow, elicits this Ca^{2+} asymmetry. However, by imaging Ca^{2+} specifically in crown cells, Yoshiba *et al.* make the surprising finding that Ca^{2+} concentrations show no L-R differences in these cells. Intriguingly, through pharmacological



Feel the flow. (A) The early mouse embryo (day 8.25), showing asymmetric expression of the indicated genes around the node and laterally. (B) The node consists of a depression containing central pit cells with motile cilia and peripheral

crown cells with immotile sensory cilia. (C) Cross section through the node showing Pkd11-Pkd2 complexes in crown cell cilia sensing leftward flow. The signaling pathway links fluid flow across the node with asymmetric gene expression.

intervention, the authors demonstrate a role for Ca^{2+} signaling in L-R asymmetry within crown cells. Thus, the links between Ca^{2+} signaling in crown cells and L-R asymmetry are still to be clarified. The asymmetrical Ca^{2+} signal (3) is likely occurring in more lateral endoderm cells, where it may play a role in the transfer of asymmetric signals from the node to the left side of the embryo (8).

Cilia are sensory organelles (9). In kidney epithelial cells, where Polycystin-1 (Pkd1) and Pkd2 localize to cilia, they act as flow sensors (10). *Kif3a* is required for cilia formation, and *Kif3a*-deficient mutant mice lack all node cilia (11, 12). When expression was rescued only in crown cells, the resulting mouse embryos lacked the central motile cilia, but still possessed the putative sensory cilia around the node periphery. Application of exogenous rightward fluid flow resulted in activation on the right side of the normally left-restricted genes. Thus, the presence of immotile cilia on the crown cells is sufficient for sensing flow in the node, strongly supporting the two-cilia hypothesis.

The results of Yoshida *et al.* suggest a model in which Pkd2, acting within crown cell cilia, is part of the leftward flow-sensing unit and that flow activates Pkd2, which in turn inhibits expression of *Cer12*. However,

Pkd2 is thought not to be a sensory protein but instead part of a complex with a sensory partner, such as Pkd1 in the kidney. A strong flow-sensing candidate protein, recently identified as critical in L-R determination in mouse and fish, is the polycystin transmembrane protein polycystic kidney disease 1-like 1 (Pkd111) (7, 13). Pkd111 forms a complex with Pkd2 in cilia (7, 13), leading to the notion that the flow-sensing complex comprises the sensor Pkd111 and the effector Pkd2.

How do the crown cell cilia sense flow? One possibility is through chemosensation of a determinant molecule that becomes enriched on the left in response to flow; another is by direct mechanosensation of the force induced by flow itself. No experiment has yet distinguished these possibilities, though circumstantial evidence supports the mechanosensation hypothesis. By analogy to Pkd1 function in kidney, Pkd111 might mediate mechanosensation in the early embryo (7). Indeed, mutation of a region in the extracellular portion of both Pkd111 and Pkd1 (called the PKD domain) abrogates function, consistent with the role of the domain in force detection (7, 14). However, the lack of detectable immotile cilia in the medaka fish Kupffer's vesicle—the functional homolog of the mouse

node—argues against mechanosensation (13). Furthermore, the left-sided enrichment of a chemical in the node is physically possible (15). A broad approach encompassing genetics, embryology, and biophysics, likely in combination with an understanding of microfluidics and physical modeling, will be required to unravel the possibilities.

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Acknowledgments: D.P.N. and D.T.G. are supported by the UK Medical Research Council.

10.1126/science.1230401

GEOPHYSICS

Seeing Is Believing

Benjamin A. Brooks

The lava lamp on my son's bureau gives him a vantage point to ponder the colorful blobs rising and falling as their temperature and density change. Geologists are not so privileged. Some of Earth's most impressive geologic features, such as the massive granitic plutons of California's Sierra Nevada mountains, are direct consequences of magma transport but we cannot directly observe these transport processes. On page 250 of this issue, Fialko and Pearce (1) use satellite data and computational modeling to infer the transport process in one location. They conclude that a magmatic diapir—a roughly spheroidal mass of partially molten material—is rising beneath a portion of the Altiplano Plateau in the central Andes.

To deduce subsurface processes, geologists must compare displacements of millimeters to centimeters per year on Earth's surface with mathematical models. However, different models can give similar predictions within the observational errors, and the most physically plausible models are often too computationally intensive to allow adequate comparison with the data. Fundamental advances thus require some combination of increased observational and computational power. In their tour-de-force study, Fialko and Pearce achieve both.

The authors used the interferometric synthetic aperture radar (InSAR) technique (2) with data from three different satellites to create an 18-year record of surface displacement at Bolivia's Uturuncu volcano. The study region sits directly above what is believed to be the largest active magma body in Earth's continental crust: the Altiplano-Puna Ultralow-Velocity Zone (APULVZ),

Satellite data and computational modeling provide evidence for a spheroidal magma body rising within the crust below the Altiplano Plateau in the central Andes.

which is ~17 to 19 km deep, ~1 km thick, and ~100 km wide (3, 4).

Previous studies, based on 8 years of InSAR data from two satellites with similar viewing geometry, identified surface uplift associated with Uturuncu and modeled it as the manifestation of an inflating magmatic point source at a depth of ~15 to 17 km (5). To better constrain the depth, geometry, and time history of the supposed magmatic source, Fialko and Pearce tasked a third satellite to acquire data from a very different look direction. Together with the previously acquired data, this more robust data set revealed an unexpected pattern of subsidence around the periphery of uplift.

The combined uplift and subsidence is difficult to explain with models that assume elastic behavior of Earth's crust. Fialko and Pearce therefore tested increasingly sophisticated viscoelastic models. Their compelling conclusion is that the APULVZ is a magma

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source for a diapir that rises toward the surface [see fig. S6 in (1)]. The rising, ballooning diapir causes the surface uplift; simultaneous withdrawal of material from the APULVZ causes the peripheral subsidence.

It is easy to agree that diapirism occurs in a lava lamp: We can see it happening. But Earth's crust consists of rock with much higher viscosity; there has been no consensus on whether diapirism is a viable magma-transport process. Objections have centered mostly on the lack of field evidence for rocks intensely deformed by passing diapirs (6).

These objections caused many to argue that silicic magmas are transported in blade-like dikes, of which there are myriad field examples, albeit with low-viscosity iron- and magnesium-rich compositions. On the other hand, more recent thermomechanical

analysis suggests that we should not discard the possibility of diapirs, because dikes with more viscous, silicic compositions may freeze within a few hundred meters from their source region (7).

Fialko and Pearce's study provides the strongest evidence to date for the existence of diapirs. The obvious next question is: How prevalent are they? InSAR studies have detected many inflation signals associated with volcanoes around the world, especially in the Andes (5) (see the figure). If each of those centers were studied as thoroughly as Uturuncu, how many would turn out to be diapir-fed?

A more time-critical question, however, is how the new findings affect hazards. A large and rising magma body sounds ominous. Uturuncu has not erupted for ~270,000

years, but the region has experienced massive eruptions during the past 10 million years (8). Recent geochemical and seismic analysis of Uturuncu provides no evidence for a shallow magma chamber that would feed an imminent eruption, and the rising magma may never reach the surface (9). Nevertheless, many parties will certainly continue monitoring the volcano.

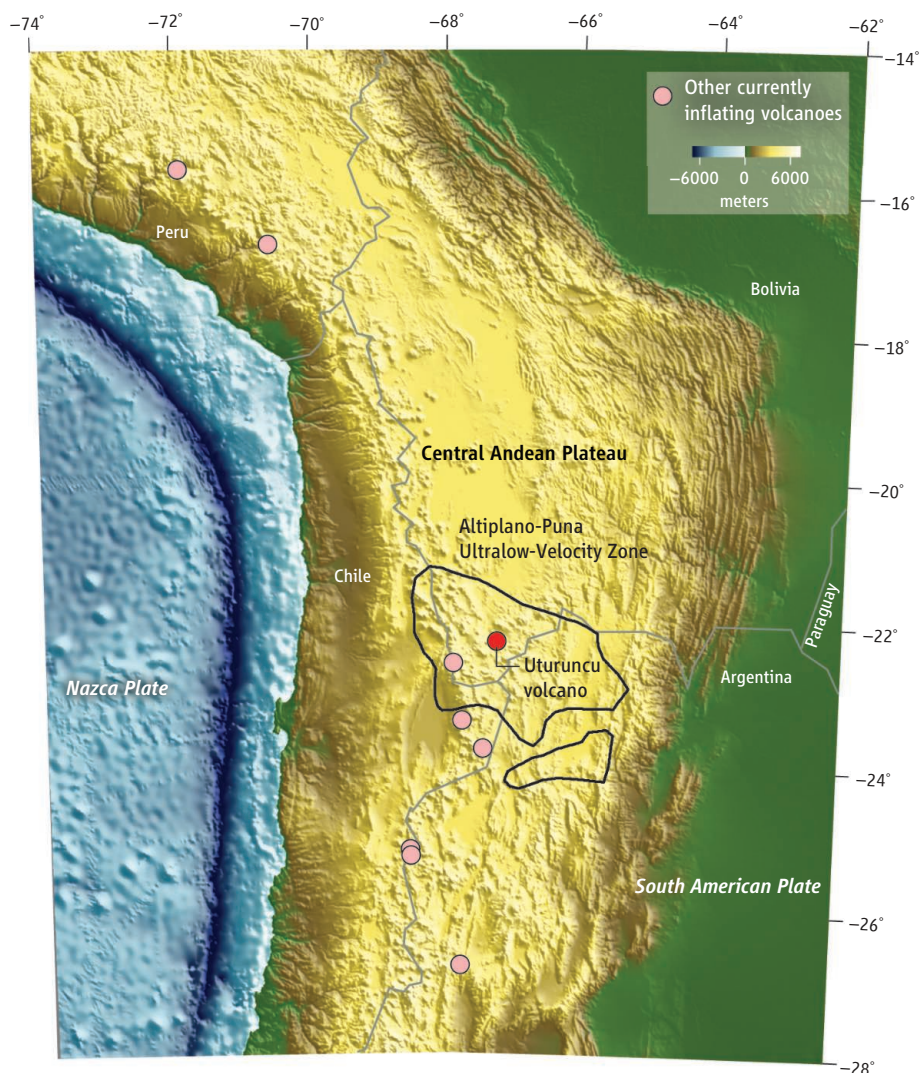
In addition to providing critical evidence to inform the magma-transport debate, the occurrence of this diapir and its connection to the APULVZ places it in the middle of another fundamental discussion about how continental plateaus achieve their height. The diapir requires a large subsurface magma body to inflate the balloon continuously over decadal time scales. But how did the large magma body get there in the first place?

A popular notion is that in the central Andes, crustal thickening due to the subduction of the Nazca Plate below the South American Plate caused the bottom of the continental crust to densify, "delaminate," and sink into the mantle (10). The isostatic adjustment from this rapid decrease in bulk density below the mountains caused the plateau to rise almost instantaneously by geologic standards (11). The thermal conditions set up by the purported delamination facilitated the APULVZ as a mid-crustal zone of partial melt (3). If the delamination hypothesis is true, diapirs could be a necessary by-product.

The potential connection of the observed diapir to delamination, itself a result of a tectonic collision, reminds us that only a few decades have passed since the theory of plate tectonics was developed. Although Earth-orbiting satellites and computer simulations have replaced ship-towed magnetometers and graph paper as the tools of the moment, we are still sorting out the ramifications of modern Earth science's defining achievement.

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Surface clues to subsurface processes. There are several currently inflating volcanoes (pink circles) in the Andes (12), including the Uturuncu volcano (red circle) above the Altiplano-Puna Ultralow-Velocity Zone [APULVZ, black line, from (4)]. On the basis of satellite data and computational modeling, Fialko and Pearce conclude that a magmatic diapir, sourced from the APULVZ, is rising below Uturuncu. It remains to be shown whether other inflating volcanoes are also fed by diapirs.

10.1126/science.1228953

INTRODUCTION

Forceful Thinking

TRANSFORMING A SINGLE FERTILIZED EGG INTO A COMPLEX ANIMAL IS A MARVEL and a mystery. Decades of study have identified molecules and mechanisms for specific steps in this transformation. But far more is involved in the construction process than the simple conversion of undifferentiated cells to a defined fate. When gazing at the stages from embryo to adult, it is clear that a multitude of cell movements and deformations (compression, extension, intercalation, and rearrangement, to name a few) are at play to shape the folds, cavities, and appendages. How do these dynamic activities come about? Biomechanics is a likely contributor, with forces exerted by the environment as well as by contractile and protrusive activities between cells. Investigators with expertise in physics and computational biology collaborate with developmental biologists to understand how forces shape animal form. *Science's* special section titled Forces in Development highlights but a few such advances in the application of physical reasoning to developmental events.

Many tissue patterns result from the interpretation of a concentration gradient of diffusible chemical substances, called morphogens, by cellular signaling cascades and transcriptional networks. As outlined by Kicheva *et al.* (p. 210), biophysical models, genetics, and quantitative imaging have been applied in the embryo to elucidate morphogen behavior and pattern readout for the coordination of events across molecular, cellular, and tissue scales. Physical reasoning has also advanced our understanding of how cells of different fate are sorted or compartmentalized. Amack and Manning (p. 212) review surface tension as important for tissue boundaries, but describe more recent work showing that adhesive molecules and mechanical polarization are also key.

Besides measuring physical elements, alternative theoretical views assist in understanding development. Furusawa and Kaneko (p. 215) discuss a dynamical systems approach to the transition between stem cells and differentiated cells. This Perspective highlights the importance of cell-cell interactions and oscillations in transcription and protein expression and is instrumental in the application of quantitative analyses to explain stem cell behavior. Finally, Newman (p. 217) notes that stereotypical morphological motifs that generate tissues that elongate, fold, and segment show similarity to the outcomes of physical processes with condensed, chemically active, viscoelastic materials. It is suggested that physical processes came into play when mobilized by ancient genes in a multicellular context for the establishment of animal form.

Technological advances have enabled the studies above, but such interest is not new. Ray Keller (p. 201) reminds us of work from the mid-19th century, when embryologists explored developmental mechanics and the forces generated by cells shaping the embryo. Going forward, the combination of technological improvements with physical and biological approaches should yield ever more clarity to understanding the biomechanics underlying development.

— BEVERLY A. PURNELL

Forces in Development

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The above series shows the dynamics that localize zebrafish sensory cells.

Science

Developmental Pattern Formation: Insights from Physics and Biology

Anna Kicheva, Michael Cohen, James Briscoe*

The spatial organization of cell fates during development involves the interpretation of morphogen gradients by cellular signaling cascades and transcriptional networks. Recent studies use biophysical models, genetics, and quantitative imaging to unravel how tissue-level morphogen behavior arises from subcellular events. Moreover, data from several systems show that morphogen gradients, downstream signaling, and the activity of cell-intrinsic transcriptional networks change dynamically during pattern formation. Studies from *Drosophila* and now also vertebrates suggest that transcriptional network dynamics are central to the generation of gene expression patterns. Together, this leads to the view that pattern formation is an emergent behavior that results from the coordination of events occurring across molecular, cellular, and tissue scales. The development of novel approaches to study this complex process remains a challenge.

In many developing tissues—from plant roots and shoots to the *Drosophila* blastoderm, imaginal discs, and the vertebrate neural tube and limbs (1)—the spatial patterns of cell differentiation are directed by concentration gradients of signals, termed morphogens (Fig. 1). Over the past few years, collaborations between biologists and physicists have begun to provide new insight into how these gradients are formed and how cells use the graded information to control differential gene expression.

Establishing Morphogen Gradients

Much attention has been focused on how morphogen profiles are established. There are examples of morphogen gradients formed by lineage transport (caused by cell growth and division) (2) or directional transport of the morphogen through a tissue (3); however, most known gradients form through morphogen secretion from a localized source, nondirectional spreading, and clearance/degradation throughout the tissue (1). Biophysically, these processes are described by the diffusion equation with production and degradation terms and defined boundary conditions. This simple description has served as the theoretical basis for developing quantitative imaging assays that measure the effective diffusion coefficient, production, and degradation rates in tissues (4–8). Reassuringly, these assays confirm that the measured rates can explain the observed gradient shapes. Nevertheless, how the effective rates arise from the amalgamation of specific subcellular processes—such as binding to receptors, extracellular matrix, and trafficking—remains less clear. This is reflected by the observation that morphogen kinetics measured with different methods at short (subcellular, seconds) (6, 9, 10) and long (tissue, hours) (4–8) space and time scales often differ.

To reconcile these differences and understand how subcellular events affect tissue-level gradient behavior, assays and analyses that connect molecular to tissue scales are necessary (11, 12). One approach has been to explicitly incorporate distinct molecular interactions into diffusion-based models. Experiments motivated by these theoretical analyses have shown that morphogen interactions with surface receptors or inhibitors that diffuse at different rates can affect gradient shape (7, 13). Such interactions and feedback can cause the effective degradation rate or diffusion coefficients to depend on morphogen concentration. This has been shown to increase the gradient robustness to fluctuations in morphogen production (14, 15).

Recent studies also recognize that gradient amplitude and/or decay length can change substantially (up to an order of magnitude) during the time course of pattern formation (16–20). In some cases, this results from increasing morphogen production from a growing source, or changes in the kinetic rates. Biophysical analysis has shown how these changes can allow the gradient shape to scale with tissue size (13, 16, 17). Understanding how dynamic gradient behavior relates to downstream interpretation and ultimately results in the precise positioning of gene expression domains is a major challenge. Is the gradient read out in a defined time-window of development (“competence period”), or do cells continuously integrate the changes in morphogen concentration? How do cells respond to stochastic fluctuations in the diffusing morphogen? Do morphogen-induced cell responses, in turn, influence the gradient shape? To address these questions, high-resolution measurements of morphogen dynamics, downstream signaling, and gene activation in individual embryos will be necessary (21).

Dynamics of Gene Regulation Control Pattern Formation

Morphogen concentration has often been seen as a direct correlate of positional identity, implying that a snapshot of the gradient is predictive of the

final tissue pattern. However, not only do ligand gradients change over time, but the intracellular signal transduction converts morphogen concentration into levels of activated transcriptional effectors in ways that can be nonlinear because of feedback or amplification (Fig. 1B) (22, 23). Moreover, the changes in signaling levels can occur on similar time scales to pattern formation (24). Therefore, signaling levels cannot be linearly extrapolated from a snapshot of morphogen concentration.

Understanding the specification of cell fate downstream of signaling also requires consideration of the gene regulatory dynamics. The signaling effectors, activated by the morphogen, control downstream target genes that cross-regulate each other to form a gene regulatory network (GRN) (Fig. 1, A and C). (For clarity, here we exclude components of the signaling cascade from the definition of a GRN, although in reality, they are part of the global GRN.) The consequence of this cross-regulation is that the expression of a target gene is not determined solely by the signaling effector but also by input from the members of the GRN.

Gene activation by even a few dynamically changing regulators rapidly becomes complex and difficult to understand intuitively. This necessitates a combination of quantitative imaging, genetics, and mathematical modeling. For example, computational models of the *Drosophila* gap-gene network comprising linked differential equations show that the experimentally observed spatiotemporal changes in gap gene expression in the syncytial embryo can occur because of the dynamics of the GRN, even when morphogen signaling is constant (25, 26). Crucially, this indicates that the gene expression profile at a specific time point depends on the previous transcriptional state. Moreover, it allows the relative strength and influence of particular GRN members to be compared with each other and the morphogen (26, 27). Similarly, in the vertebrate neural tube the dynamics of a small transcriptional network downstream of Shh signaling gives rise to the observed changes in tissue pattern in a way that cannot be explained by the dynamics of the signaling effectors alone (28).

At present, only small subnetworks of about six or fewer genes have been explicitly mathematically modeled in order to investigate how temporal changes in cell identity arise from both morphogen signaling and the activity of a GRN (Fig. 2). However, tissues can be composed of many (for example, at least 14 in the spinal cord) spatially separated distinct cell identities defined by combinatorial gene expression of an unknown number of genes, linked in a global network. Although detailed connectivity maps of these large networks are not yet available, it has been proposed that much of the global GRN function can be understood by breaking it down to network substructures that function quasi-autonomously (29, 30). Although the validity of this approach remains unclear, the recent studies demonstrate that small networks successfully explain pattern

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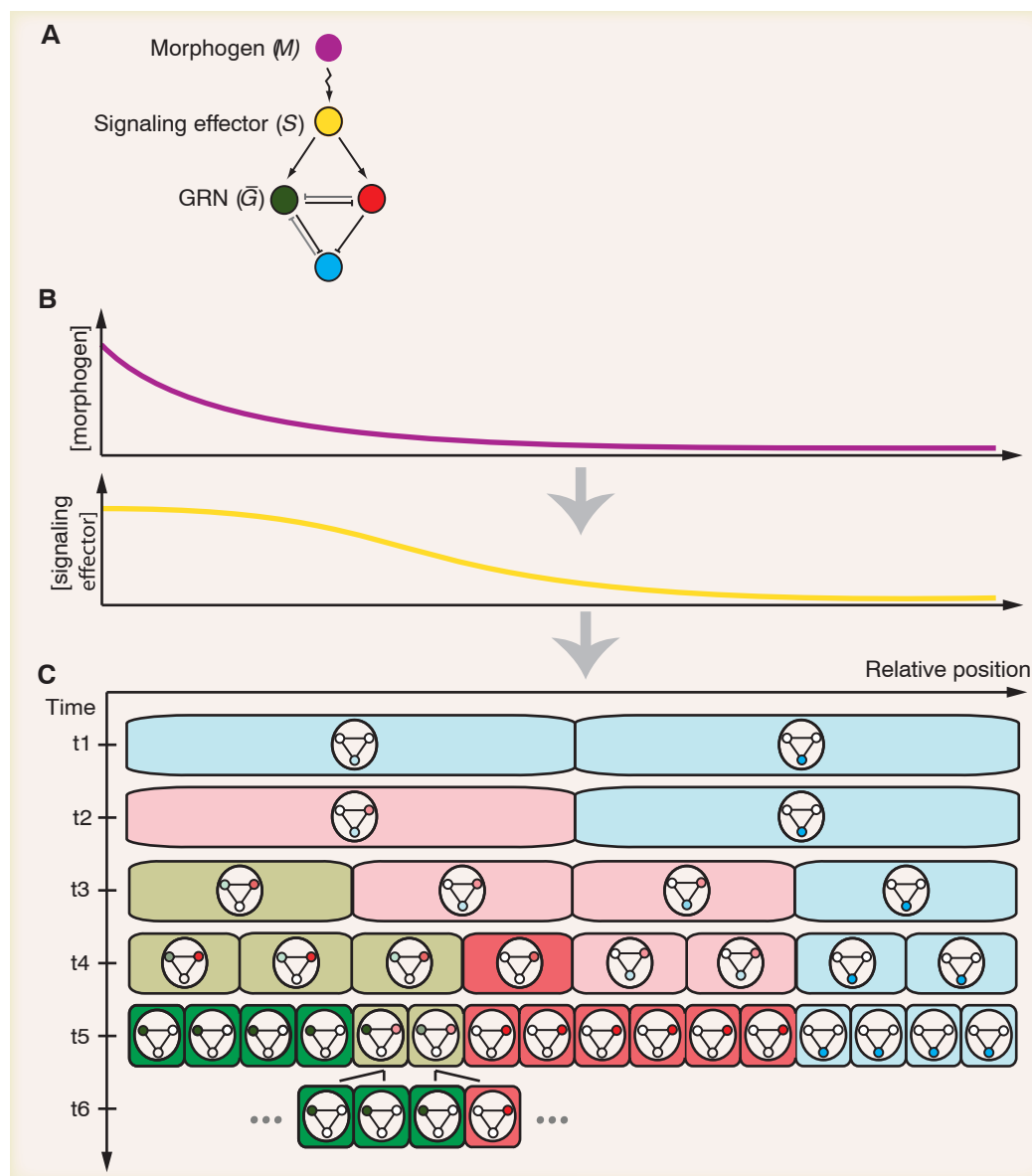


Fig. 1. (A) Cells respond to a morphogen (purple) by activating signaling effectors (yellow), which induce some of the GRN genes (blue, green, and red). (B) Morphogen gradients are transduced nonlinearly into signaling gradients. (C) The signaling gradient and GRN interactions cause dynamic changes in gene expression levels (shades of network nodes) in each cell. The cell state (outer color) is determined by the combination of active genes (nonwhite nodes). The number of cells increases by proliferation; for clarity, a normalized field is shown. In the simplified situation depicted here, the initially identical cells are exposed to a time-invariant signaling gradient. A changing gradient would add another layer of complexity to the patterning process.

in part of an organ (such as the ventral neural tube, or the central region of the fly embryo) (26, 28). In addition, the identification of synthetic networks containing only a few genes that generate patterns in response to morphogens supports this view (31, 32). Finding these subnetworks has implications not only for understanding positional information, but also opens tractable ways of addressing questions relating to the evolvability and adaptability of morphogenetic control.

Moreover, detailed dynamical models of small networks reveal how they allow cells to buffer

fluctuations in morphogen concentration and maintain a transcriptional state (28, 33). The positional variation in morphogen concentration empirically appears to be larger than that of gene expression boundaries (19, 34, 35), and studies have shown that transcriptional networks can enhance the precision of patterning (33). However, the effect of transcriptional noise on the precision and reliability of patterning is less well understood. In part, this is because stochastic models are difficult to configure, and realistic noise measurements require *in vivo* assays with high temporal and spatial resolution.

small systems with defined components; models of larger networks rapidly become complex, computationally cumbersome, and difficult to parameterize and test.

Alternative approaches attempt to reduce the complexity of large networks by identifying minimal subnetworks of core regulator genes (39) that are sufficient to predict the pattern of many genes in the tissue. Other alternatives use geometric representations of dynamical systems in which cell identities are treated as attractors in a multidimensional state-space (Fig. 2B) (40, 41). In this view,

Specific subnetworks might also explain how cells read time derivatives and spatial derivatives of graded signals (16, 36, 37). These functions are particularly relevant for the regulation of tissue growth by morphogens, and it is an open question how they might be involved in coordinating growth with cell fate specification. Existing mathematical models of morphogen-driven subnetworks that define cell identity mostly consider a constant signaling input and do not account for growth. To simulate more realistic scenarios of how a changing morphogen concentration is transformed into a specific cell fate, there is a need for measurements to compare the dynamics of local signaling, target gene expression, changes in the position of cells within tissues due to growth, and the inheritance of transcription factors and signaling molecules during cell division.

Toward a New Definition of Positional Information

Together, these recent studies indicate that spatial patterns of cell identity emerge from the dynamic interaction of ligand gradients, signaling effectors, and transcriptional networks (Fig. 1), all of which appear to change on similar time scales. In this view, there is no simple molecular correlate of positional identity (38): The spatial pattern of cell identities over time is dynamically determined by the morphogen signaling levels and depends recursively on the transcriptional state of cells. Precisely how these different processes are coordinated to achieve reproducible patterning outcomes is not yet clear. A current limitation is that mathematical models that describe the detailed molecular processes underlying GRN and gradient dynamics (Fig. 2A) can only realistically be applied to

each dimension is chosen to represent some measurable variable, such as the level of activated signaling effector or a regulator gene. Experimentally observed temporal changes in cell state are mathematically described as trajectories through this state-space (33, 40). Such models could provide quantitative insight into the relationship between regulation and cell identity and do not necessarily need information on the underlying genetic network (40). However, they require cell states to be empirically determined and are difficult to relate directly to a molecular mechanism. Nevertheless, it might be possible to use such approaches in conjunction with other methods from statistical physics. These could include the detailed examination of noise transmission and characterizing the information-processing capacity of systems (21, 42).

An evident challenge will be matching analytical frameworks to experimental data and integrating the analyses across time and length scales so that molecular and cellular mechanisms can be linked to tissue-level behavior. For these reasons, a continuing dialogue between theoreticians and experimentalists will be necessary.

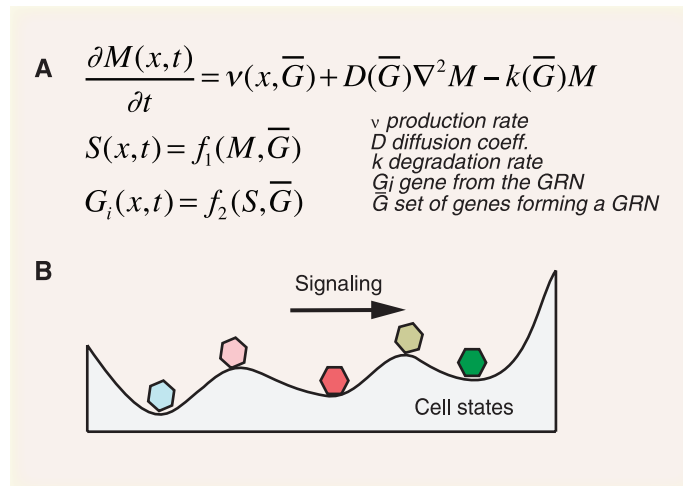


Fig. 2. (A) Generic dynamical model of the system in Fig. 1. Morphogen (M) gradient formation is described by a diffusion equation. The degradation rate k , diffusion coefficient D , and production rate v may be dynamically altered by feedback from GRN ($\bar{G}$) genes. The signal S is a function of M and $\bar{G}$. The expression of each target gene (G_i) is determined by the GRN and S . **(B)** Geometric description of the system showing possible transient (saddle points) and stable (depressions) cell states (colors are as in Fig. 1C). The transitions between states depend on the levels and duration of signaling. A one-dimensional representation is shown for clarity, but multidimensional state-spaces follow a similar rationale.

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Acknowledgments: We thank B. Baum and Manu for helpful comments. Funding provided by the Medical Research Council (UK).

10.1126/science.1225182

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REVIEW

Knowing the Boundaries: Extending the Differential Adhesion Hypothesis in Embryonic Cell Sorting

Jeffrey D. Amack^{1*} and M. Lisa Manning^{2,3*}

Successful embryogenesis requires proper sorting and compartmentalization of different cell types. Mechanical interactions between cells help govern these processes. In the past, physics-based theories have guided in vitro studies of cell sorting and tissue surface tension. Recent experiments have challenged this approach, indicating that adhesive molecules also act as signaling molecules that initiate local reorganization of actomyosin and demonstrating that cells at the boundary of a colony of initially identical cells become “mechanically polarized.” Extending physical models to account for mechanical polarization helps solve a long-standing paradox about magnitudes of tissue surface tensions and potentially explains discrepancies between recent in vivo and in vitro cell-sorting experiments. New experiments are needed to further explore the connection between mechanical polarization and tissue boundary formation in vivo.

How do cells in a developing embryo know where to go and how to behave once they get there? Sometimes a programmed

chemical signal, called a morphogen, guides cells, whereas in other cases, cells self-organize. Cell sorting and tissue layering behaviors are

remarkably robust: Two types of dissociated embryonic tissues that are homogeneously mixed will spontaneously sort into layers. But how do cells know which way to sort?

The Differential Adhesion Hypothesis

Physical reasoning has provided a partial answer. More than 50 years ago, Steinberg proposed the differential adhesion hypothesis (DAH), which postulates that (i) each type of embryonic tissue has a unique “tissue surface tension” (TST) that governs how tissues sort, just as fluid surface tension governs how immiscible liquids demix, and (ii) differences in TST arise from differences in cell-cell adhesion (1). Because interactions between cells are often difficult to probe inside an embryo, investigations of the DAH have largely been carried out *in vitro*. By measuring forces applied to cellular aggregates *in vitro*, one can extract a surface tension-like quantity and demonstrate that “effective surface tension” accurately predicts sorting behavior. However, key questions about the DAH remain unresolved: How do single-cell properties such as adhesion and cortical tension impact TST? How do *in vitro* analyses relate to tissue layering and compartmentalization in a developing embryo?

The answer to the first question is simple if cells in a tissue are just like molecules in a fluid, where the surface tension depends on the depth of the interaction potential between the molecules (their “adhesion energy”) and the area over which the molecules interact. In this scenario, the TST is roughly equal to the adhesion energy of a cell per unit area. Because adhesion in tissues is predominantly mediated by cadherin molecules, the TST should equal the energy required to rupture a cadherin bond times the number of cadherins per unit cell surface area. Experimental studies have shown that TST is indeed proportional to the number of surface cadherins (2).

However, the magnitude of TST measured in cell aggregates—about 1×10^{-3} N/m—does not

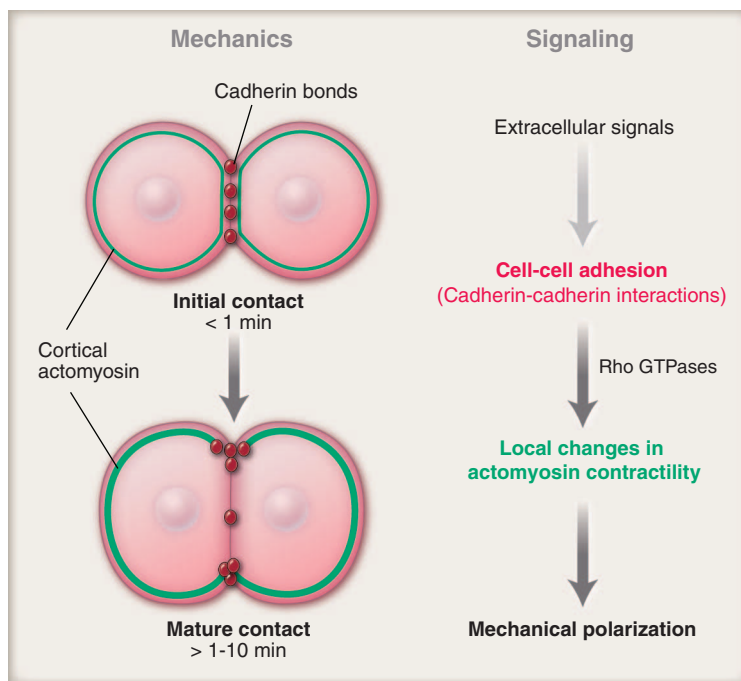


Fig. 1. Mechanical polarization in cell doublets. Initially, two cells coming into contact will adhere via the external domains of cadherin molecules (red dots). Over longer time scales, internal domains of cadherins interact with actomyosin, which leads to the reorganization of actin cortical network (green lines) in a mature cell-cell contact. Recent work demonstrates that this often leads to up-regulation of cortical tension along the external boundary and/or down-regulation along internal interfaces. It may be that upstream extracellular biochemical signals, such as Hedgehog, influence this cascade.

support this idea. The rupture energy of cadherin bonds is about 10 kT or 1×10^{-19} Joules, and there are about 10 cadherins per square micron on the surface of typical cells (3), which together result in an adhesion energy per unit area of about 1×10^{-7} N/m, which is four orders of magnitude smaller than typical TSTs. Other experimental results (3, 4) support theories (5, 6) that hypothesize that cell sorting correlates more strongly with the magnitude of single-cell “cortical tension” generated by active actomyosin contractility rather than cell adhesion.

Mechanical Polarization

These apparently contradictory conclusions are based on the assumption that all cells in an undifferentiated tissue are roughly identical and can be characterized by properties measured for a single cell. Several recent papers point toward an intriguing possibility for resolving these contradictions because they demonstrate that cells at tissue boundaries are different from those in the interior. They suggest that boundary cells actively change their mechanical properties or “mechanically polarize.”

Building on previous work (7, 8), Gardel and co-workers have developed an exciting new traction force imbalance method (TFIM) that determines exact forces exerted across cell-cell contacts

in small groups of adherent cells on two-dimensional (2D) substrates in unconstrained geometries (9). Unlike laser ablation (10) or pipette aspiration (11) experiments, TFIM makes no assumptions about the mechanical properties of cells. TFIM was used to quantify mechanical polarization in cell pairs and triplets: Cells reorganized their adhesive and cytoskeletal machinery so that tension forces and actin density were substantially higher along external interfaces of doublets or triplets than on internal interfaces.

Extending these ideas to larger cell populations, Mertz *et al.* (12) demonstrate that coherent colonies of initially identical cells on 2D surfaces can be characterized by an effective surface tension. Just as in the Gardel experiments, traction forces were localized near the boundary of the colony, and cells at the boundary were mechanically polarized. Another recent study by Krens *et al.* (13) showed that cells on the surface of 3D zebrafish embryonic explants develop strong apical-basal actin polarization, although these cells express epithelial markers at later times and may also be differentiated.

These results show that many different cell types in different environments have machinery to sense and respond mechanically to “being at a boundary.” What are the components of this machinery? An intriguing possibility is that cadherins act as signaling molecules, altering local actomyosin dynamics and generating cell polarization (Fig. 1). Earlier work on cell doublets has shown that cadherin-mediated adhesion regulates Rho family guanosine triphosphatases to reduce actomyosin contractility along a cell-cell contact interface relative to the edges of the contact (14), which is consistent with the TFIM experiments (9). Generalizing to larger aggregates, one would expect cortical tension to be reduced at all cell-cell contacts for cells in the middle of a confluent tissue. In contrast, cells at the boundaries would have a reduced cortical tension only along internal “contacting” interfaces, which could lead to mechanical polarization of boundary cells similar to that seen in experiments (12). So, although the mechanical energy of cadherin bonds is very small, cadherin signaling might still be responsible for boundary polarization.

An Extended Differential Adhesion Hypothesis

These results also demonstrate that “surface tension” is a robust property even in tissues or

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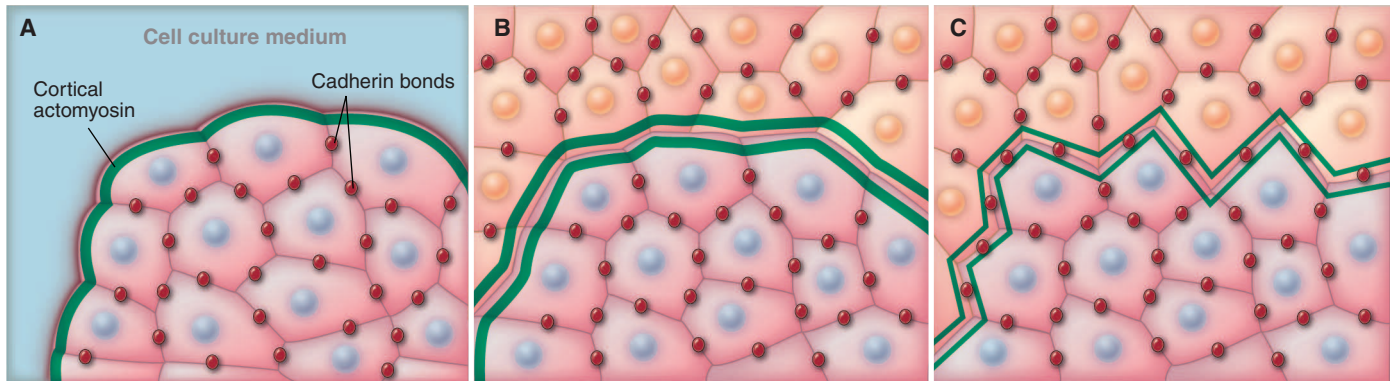


Fig. 2. Mechanical polarization at tissue-culture and tissue-tissue boundaries. Green lines indicate a higher-than-average density of cortical actomyosin, and the thickness of the lines indicates the amount of tension generated. Red dots indicate multiple cadherin bonds. (A) Many tissues have cells that mechanically polarize at interfaces with culture medium. This effect can explain the

magnitude of TST in vitro. (B and C) Nuclei are labeled blue or orange to designate two different tissue types. Even if boundary polarization occurs for individual tissues in vitro, it may (B) or may not (C) occur at the interface between two tissues; this may explain differences between in vivo cell intercalation and in vitro cell sorting.

colonies where cells are locally and actively changing their mechanical properties. Simple models that express a cell's mechanical energy as a function of cortical tension, adhesion expression, and elasticity can easily account for mechanical polarization at boundaries. They accurately describe shapes of groups of cells in culture and in epithelial layers in vivo, even in the presence of fluctuations and cell divisions (11, 15). For these tissue types, it is reasonable to hypothesize that TST is the mechanical energy penalty incurred in increasing tissue surface area by moving cells to the boundary.

From this type of model, it has recently been shown that, if cells at the boundary mechanically polarize, the net mechanical effect on the tissue is the same as if there were extra adhesion between all of the cells (16). Within an extended DAH framework, TST is then proportional to an "effective adhesion" that is the sum of cadherin adhesion energy, about 1×10^{-7} N/m, and a term equal to the difference between cortical tensions along internal and external interfaces, about 1×10^{-3} N/m (11, 17). This suggests that TST is dominated by boundary mechanical polarization instead of the mechanical energy of adhesive bonds. And, if mechanical polarization is regulated by cadherin signaling, this could explain why TST scales with the number of surface cadherins and yet why it is strongly correlated with actomyosin-dependent cortical tension.

Mechanical Polarization in Vivo

Evidence for boundary mechanical polarization from in vitro studies provides a starting point for formulating hypotheses about tissue layering in an embryo. Boundary polarization certainly occurs in vivo. For example, recent work in *Drosophila* has demonstrated that actomyosin cables are critical for maintaining compartment boundaries in the embryo (18), and clonal cells with increased adhesion generate an acto-

myosin cable at the clone interface (19). But are the same mechanisms that regulate mechanical polarization between cells in culture involved in establishing tissue boundaries in embryos?

Little has been done to determine how properties that are important for cell sorting in vitro affect cell sorting in vivo. Recent work by Ninomiya *et al.* demonstrates that tissue intercalation and compartmentalization in vivo does not always correlate with in vitro cell sorting or TST measurements (20). Their results suggest that although in vitro sorting is dominated by short-time scale interactions between the external domains of adhesion molecules, in vivo intercalation is dominated by long-time scale spatial reorganization of cadherins and cortical tensions. Ninomiya's in vivo work in the *Xenopus* embryo focused on dense, intercalating tissues, where cell-cell contacts are mature and long-lived. Other researchers have focused on in vivo processes, where cells are less densely packed and change neighbors much more quickly, such as germ-layer progenitor cells during the shield stage in zebrafish (21, 22), where the short-time scale adhesive interactions that govern in vitro sorting are more likely to be important.

Boundary mechanical polarization might provide a framework for explaining Ninomiya's results and uniting in vivo and in vitro studies. If surface tension is dominated by long-time scale boundary polarization, the cadherin density should not correlate with surface tension when cadherins are missing internal domains that interact with the cytoskeleton, as observed. Furthermore, it is tempting to speculate that in vivo interfaces with intercalating cells are ones that have not mechanically polarized, even though each individual tissue boundary polarizes in cell culture medium (Fig. 2). This could explain the discrepancy between differential TST and cell intercalation. Finally, cells in vivo often interact with complex extracellular matrix structures, which

might influence boundary polarization and cell sorting.

Looking Forward

To test these hypotheses, new experiments and techniques are needed to (i) analyze cytoskeletal organization along tissue boundaries, (ii) quantify interfacial tensions and forces in vivo, and (iii) study signaling upstream and downstream of mechanical polarization.

It would be useful to demonstrate conclusively that mechanical polarization occurs in 3D tissues composed of identical, undifferentiated cells, just as in the 2D experiments. Fluorescent probes and confocal microscopy could be used to image actin and myosin reorganization at the boundary of a cell aggregate. The same tools could be used to investigate mechanical polarization at the boundaries between two tissue types in vivo and in vitro. Are actin and myosin more dense at an interface between two tissue types? If so, is cell intercalation less likely at a two-tissue interface that has mechanically polarized? How is actomyosin reorganization at two-tissue interfaces different from that at tissue-cell culture medium interfaces?

We also need to develop tools to precisely quantify interfacial tensions inside tissues. Laser ablation is commonly used to estimate tensions in 2D epithelial layers, but it can only do so up to an unknown constant and faces significant challenges in 3D tissues. An alternate approach would be to use TFIM to quantify interfacial tensions between two tissue types and to determine whether these tensions can be predicted on the basis of actomyosin or cadherin dynamics imaged by using confocal microscopy. Other promising new techniques include molecular force sensors (23) and membrane fluctuation analyses (24).

Experiments are needed to see whether extracellular signals regulate mechanical polarization (Fig. 1). Of the several signaling pathways

known to be involved in tissue boundary formation in vivo (25–28), Hedgehog signaling is especially intriguing because it is known to regulate myosin activity and to induce cadherin expression at tissue boundaries in *Drosophila*. Does modulating Hedgehog signaling affect actomyosin accumulation during boundary formation? It will also be critical to characterize downstream effects of mechanical polarization. Polarized cytoskeletal activity will likely result in changes in cell morphology and could provide a cue for cells to differentiate in response to their location at tissue boundaries.

Although physical reasoning can be useful in developmental biology, embryonic tissues test the limits of physical theories for collective phenomena and pattern formation. For example, it is clear that localization and transport of adhesion molecules play an important role in vivo (20). We need to develop a model that predicts how mobile adhesive molecules affect cell shapes and/or forces and vice versa. We also must extend these models to account for a broader range of mechanical effects at boundaries, such as differences in cell protrusivity and oriented cell divisions. Another challenge is to interpolate between short-time scale mechanical interactions that are important in tissues with highly mobile cells and long-time

scale interactions that are important for dense tissues with mature contacts.

Collective mechanical interactions provide a robust mechanism for dictating cell movements, and new work suggests that actomyosin reorganization at boundaries is important for these mechanical interactions in vitro and possibly in vivo. Signaling pathways upstream of this reorganization may provide the tight regulation that is critical for proper embryonic development.

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Acknowledgments: The Amack lab is supported in part by a grant from NIH (R01HL095690). The Manning lab acknowledges support from the College of Arts and Sciences at Syracuse University.

10.1126/science.1223953

PERSPECTIVE

A Dynamical-Systems View of Stem Cell Biology

Chikara Furusawa¹ and Kunihiko Kaneko^{2*}

During development, cells undergo a unidirectional course of differentiation that progressively decreases the number of cell types they can potentially become. Stem cells, however, keep their potential to both proliferate and differentiate. A very important issue then is to understand the characteristics that distinguish stem cells from other cell types and allow them to conduct stable proliferation and differentiation. Here, we review relevant dynamical-systems approaches to describe the state transition between stem and differentiated cells, with an emphasis on fluctuating and oscillatory gene expression levels, as these represent the specific properties of stem cells. Relevance between recent experimental results and dynamical-systems descriptions of stem cell differentiation is also discussed.

More than half a century ago, Waddington proposed an epigenetic landscape (1) to describe the cell differentiation process as the trajectory of a ball into branching valleys, each of which represents a developmental state (Fig. 1A). The height of the troughs

represents the potential barrier for escaping the corresponding state (2, 3), and the potential final destinations of the ball (cell) correspond to different cell types (4). A possible link between developmental dynamics responsible for this cell differentiation and a network of gene regulations has been discussed (3, 5). Stem cells, however, can both robustly proliferate (same valley) and differentiate (switch valleys). This characteristic, which is a core property of “stemness,” cannot easily be described by Waddington’s landscape. In this Perspective, we consider past dynamical-

systems approaches for cell differentiations, and then examine recent advances that go beyond this simple landscape, to describe both stem and differentiated cells.

Cells contain many components, including genes, proteins, and metabolites. The cellular state at a particular time can be represented as a point in multidimensional state space in which each axis represents the abundance of a component (Fig. 1B). Gene (or protein) expressions are a major part of such components (for simplicity, we write “gene expression level” to describe the abundance). Interplay among genes, such as the activation and repression of gene expressions, causes the cellular state to shift, a phenomenon that can be depicted as a trajectory in the state space. Temporal changes in the expressions restrict the cellular state to a certain region, which is defined as an “attractor” in dynamical-systems theory (6). After a slight perturbation (change in gene expression levels), a state returns to its original attractor with the aforementioned temporal change. The attractor can be in a fixed state over time (i.e., fixed-point attractor) where the synthesis and degradation of each product are balanced, or a set of dynamically changing states with temporally oscillating gene expressions (e.g., orange trajectory in Fig. 2B). A system can have multiple attractors of different composition. Each attractor then can be regarded as a distinct cell type (7, 8) corresponding to the different valleys into which a

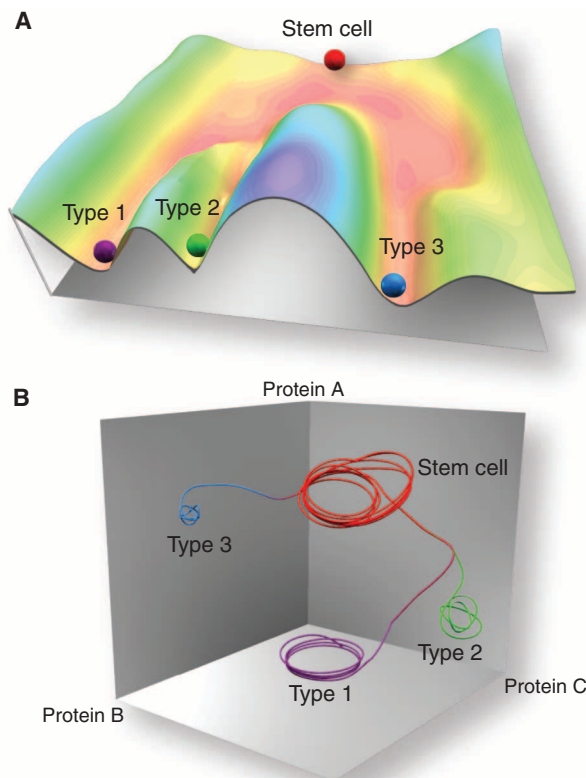
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Fig. 1. (A) Waddington's epigenetic landscape. The development of a cellular state is represented by a ball rolling down a landscape of bifurcating valleys, each representing different cell types. **(B)** Dynamical-systems representation of cellular states. Each axis represents the expression of a protein whose time development is depicted as a trajectory in space. Final states are attractors and correspond to distinct cell types.



ball can fall in Waddington's landscape (Fig. 1A). For example, when two genes, A and B, mutually suppress each other's expression (known as a toggle switch), two fixed-point attractors form: one with activated A and suppressed B, and the other with activated B and suppressed A (Fig. 2A). These two attractors can be regarded as two distinct cell types.

In addition to the above intracellular dynamics, gene expression is subject to noise-generated random fluctuations. Attractors, however, are robust to such noise, since after a perturbation the cellular state returns to its original attractor. Hence, attractors can explain robust, distinct cell types. However, this creates a paradox inherent to a stem cell's expression dynamics: A cell needs to be robust to perturbations to retain proliferation and at the same time be sensitive to perturbation to differentiate into other cell types. Two possible explanations for this property are discussed below.

The first approach focuses on the noise-induced transition between attractors. Huang introduced the self-activation of two genes into the above toggle switch (see Fig. 2A) (9). The result is the emergence of another attractor that weakly expresses both A and B. Although this attractor is robust to tiny expressions of noise, sufficiently large noise can switch it (and the

cellular state) to either an A-activated or a B-activated attractor, which is more robust against noise. Huang therefore proposed that a cell with a balanced expression of A and B could be regarded as a multipotent stem cell. In fact, this regulatory architecture contributes to the binary fate of stem and progenitor cells such as common myeloid progenitors (mutual inhibition of GATA1 and PU.1) and embryonic stem cells (Oct4 and Cdx2) (9). Noise-induced transitions between attractors have also been examined in more complex regulatory networks, since the pioneering study by Kauffman (7, 10).

However, if differentiation were driven only by noise, it would be unlikely that the loss of differentiation potency follows a deterministic course or that development is robust. A second approach notes that development involves an increase in the number of cells communicating via intracellular signaling, a property essential for maintaining distinct cell types and their homeostasis. For example, the fate of hematopoietic stem cells is regulated by intracellular signaling within

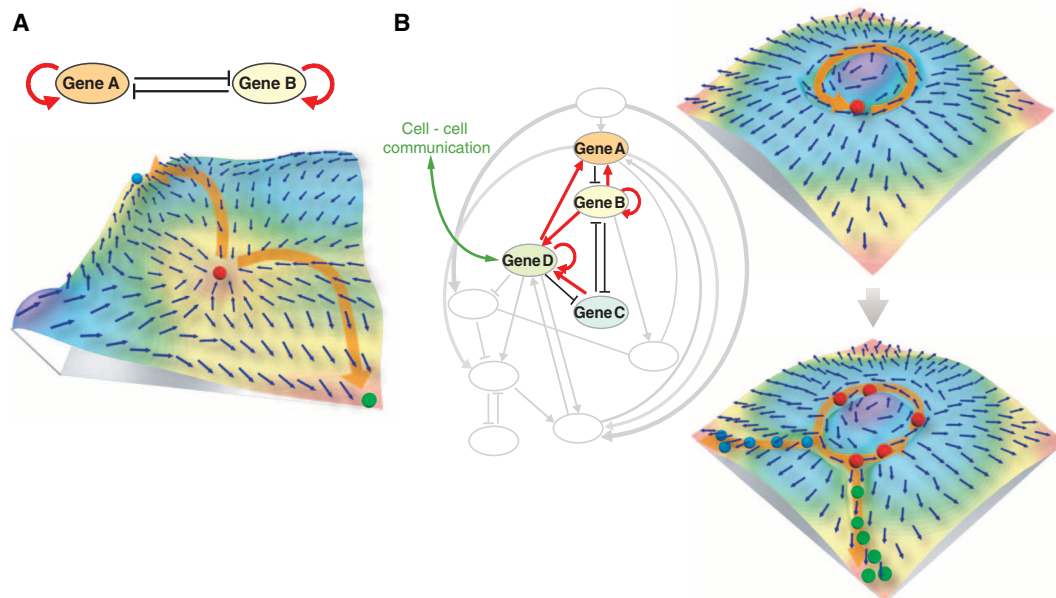


Fig. 2. Dynamical-systems view on the differentiation dynamics of a stem cell. (A) By adding self-activation (top, red arrows) to a toggle-switch network, i.e., two mutually repressing genes (9), (bottom) an attractor (red) with balanced expression of the two genes is added between A-activated and B-activated attractors (green and blue, respectively). Differentiation from the balanced expression to either of the biased attractors is triggered by noise. **(B)** Oscillatory gene expression dynamics (upper right: circulating trajectory shown by an orange arrow) are generated by negative feedback in the regulation network (left, red and black arrows). Cell differentiation is driven by cell-cell communication (green arrow) and fixed through positive feedback in the network (11, 12). An increase in cell number results in some cells leaving the original attractor (stem cell state) to differentiate, whereas those that remain proliferate (lower: orange trajectory bifurcates owing to cell-cell interactions). Orange and blue arrows on the landscape represent the trajectory of cellular state and the gradient of the landscape that affect the movement of the ball, respectively.

a niche (11). Theoretical models that include both cell-cell communication and intracellular expression dynamics have been investigated (12, 13). Extensive simulations of such models over a huge variety of gene expression networks have found that cells that can both proliferate and also differentiate to cell types of different composition generally show temporal oscillations in their gene expressions at the single-cell level (Fig. 2B). In such cases, with the increase in cell number, state differences between cells are amplified by cell-cell communication such that the sensitivity to a signal increases. Some cells at a certain phase of oscillations (i.e., at a certain location within the orange trajectory in Fig. 2B) escape their original attractor in response to a signal and fall into the trough of a different attractor, whereas other cells of different phases remain with the original attractor. Thus, gene expression oscillations are necessary for stemness, potentiality both to proliferate and differentiate, whereas the loss of stemness is characterized by a loss of oscillatory dynamics. Notably, in this mechanism, the timing and pathway of differentiation are robust to noise, a property Waddington termed homeorhesis (1). With cell-cell communication, the differentiation frequency of a stem cell is autonomously regulated by the population of each cell type, resulting in a robust population ratio.

Recently, Huang used time-series transcriptome data to experimentally verify the existence of attractors in the dynamics of hematopoietic progenitor cells by demonstrating the robustness of the cellular state (14). Additionally, from the

fluctuating expression level of stem cell marker Sca1, they found slow-scale changes in cellular states, which was suggested to be regulated by cell-cell communication (15).

Single-cell measurements of gene expression dynamics have shown heterogeneous gene expressions of Rex1, Nanog, and Stella in embryonic stem cell populations (16) and Sca1 in hematopoietic stem cells (15, 17), a heterogeneity closely linked to the fate of the stem cell. One possible mechanism for such heterogeneity could be noise in the expression dynamics. Another is oscillatory expression dynamics. Indeed, Kageyama and colleagues found temporal oscillations in the Hes1 expression level of neural precursors and embryonic stem cells, where the phase of the oscillation was potentially seen to control the fate decision (18, 19), whereas existence of a complex dynamic attractor is also suggested (20). Furthermore, cell-cell communication via Notch-Delta signaling was suggested to regulate the fate decision of neural progenitors under the control of the oscillatory expression dynamics of Hes1 and other genes (18).

Using a dynamical-systems approach to explain the differentiation of stem cells, we have described here how fluctuating and oscillatory gene expressions underlie the essence of stemness. If so, reactivating specific genes may recover these oscillations in differentiated cells to potentially restore potency (21). To characterize the attractors of stem and differentiated cells quantitatively, however, further experiments, including systematic sensitivity analysis of gene expres-

sions (22), as well as theoretical formulations that go beyond Waddington's epigenetic landscape, are needed.

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10.1126/science.1224311

PERSPECTIVE

Physico-Genetic Determinants in the Evolution of Development

Stuart A. Newman

Animal bodies and the embryos that generate them exhibit an assortment of stereotypic morphological motifs that first appeared more than half a billion years ago. During development, cells arrange themselves into tissues with interior cavities and multiple layers with immiscible boundaries, containing patterned arrangements of cell types. These tissues go on to elongate, fold, segment, and form appendages. Their motifs are similar to the outcomes of physical processes generic to condensed, chemically excitable, viscoelastic materials, although the embryonic mechanisms that generate them are typically much more complex. I propose that the origins of animal development lay in the mobilization of physical organizational effects that resulted when certain gene products of single-celled ancestors came to operate on the spatial scale of multicellular aggregates.

Many of the classic phenomena of early animal development—the formation and folding of distinct germ layers during gastrulation, the convergence and extension movements leading to embryo elongation,

the formation of somites (paired blocks of tissue) along the main axis of vertebrate embryos, the generation of the vertebrate limb skeleton, the arrangement of feathers and hairs—have been productively analyzed by mathematical and com-

putational models that treat morphological motifs as expected outcomes of physical process that are generic; i.e., pertaining as well to certain nonliving, chemically active, viscoelastic materials (1–4). Given that the thousands of genes of extant animals have been subject to mutation and (at the organismal level) natural selection over the more than 600 million years since the Metazoa first emerged (5), it is counterintuitive but revealing that the morphological motifs animals began with were carried over to the present, with few additions.

Many developmental events that might be characterized by their simple generic physical properties are, in fact, much more complex. For example, many cells of embryonic tissues are individually mobile while, at the same time, collectively cohesive, as in the formation of distinct layers during gastrulation and of boundaries during later development: behaviors that had been attributed to cell adhesive differentials, with analogy to the phase separation of liquids such as oil and water (1). Although differential adhesion is indeed capable of sorting cells into separate

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layers, the embryo is more complicated; for example, with tension being exerted on the cell surface by the cytoskeleton and active cell-cell repulsion (phenomena with no known counterparts in liquids), often contributing more to the configuration of the separated tissues than relative affinities (6)

More generally, cells in embryos have the ability, via contractile and protrusive activities, to exert forces on one another and on the extracellular matrices they produce (7). Although these mechanical properties can lead to, and in some cases account for, the buckling of epithelial tissues into ridges, as in neurulation, this developmental process actually occurs by several different mechanisms across chordates, only some of which depend on mechanically mediated buckling (8).

An embryo's cells are tiny chemical reactors with stored and exchangeable sources of energy. This is evidenced in their ability to switch among multiple stable compositional states (the basis for cell differentiation) (1, 9) and to exhibit biochemical oscillations (the basis of the cell cycle and other cell-physiological periodicities) (10, 11). By virtue of this dynamicity, embryonic tissues are chemically "excitable media," the physical properties of which can explain some enigmatic developmental phenomena. Nonliving chemical oscillators that are weakly coupled readily come into synchrony (12). Correspondingly, interactions between adjoining cells in an embryonic tissue will synchronize intracellular oscillations; an example is the periodic expression of the transcriptional modulator *Hes1* transforming a clump of individual cells into a globally coordinated "embryonic field" (13).

Although a spatial uniformity of biochemical state can thus emerge in embryonic tissues, patterns can also form based on the self-organizing capabilities of interacting diffusible activators and inhibitors of cell differentiation ("morphogens") (14–16). Some periodic and quasiperiodic developmental patterns (such as the distribution of hairs, pigment patches, or skeletal structures) clearly depend on such effects (17), but others, such as the seven stripes of pair-rule proteins in the syncytial *Drosophila* embryo, although they exhibit some self-organizing aspects (18), are generated in a less generic fashion, employing stripe-dedicated duplicated gene promoters (19).

The operation of generic physical effects in animal embryogenesis, along with developmental mechanisms that are complex and nongeneric but nonetheless produce similar stereotypical morphological motifs (multiple layers, interior cavities, segments, folds, etc.), suggest a scenario in which the nongeneric mechanisms are evolved embellishments of the generic ones, with selection stabilizing and reinforcing inherent forms rather than inventing new ones (20). Hierarchical programs of gene expression during the development of modern animals (21) regulate shape and form by coordinating, fine-tuning, and constraining the activities of a subset of the conserved developmental "tool kit," the tools of which are the products of genes that directly mediate cell-cell interactions (22). These molecules (such as cadherins, Notch, Wnt, Hedgehog, bone morphogenetic protein, and collagens) typically served single-cell functions in one or more unicellular ancestors of the multicellular animals

before being recruited into developmental roles as multicellularity emerged (23, 24).

The morphogenetic and patterning functionalities that arose when "interaction tool kit" molecules, acting in the new multicellular context, mobilized generic physical effects, have been termed dynamical patterning modules (DPMs) (22). Although primitive metazoan-type body plans could have quickly arisen in aggregates of genetically variable cells as long as they contained DPM-enabling genes (Fig. 1, aggregation route), without enforced genetic uniformity among the cells of multicellular forms, intraorganismal competition would tend to undermine their persistence (25). The emergence of an egg stage of development, with the cell cluster stage of development then generated by cell cleavage, would have obviated such chimerism (Fig. 1, cleavage route), facilitating the generation of evolutionarily stable lineages (26) (Fig. 1).

The early products of DPMs would have borne the generic morphological signatures of

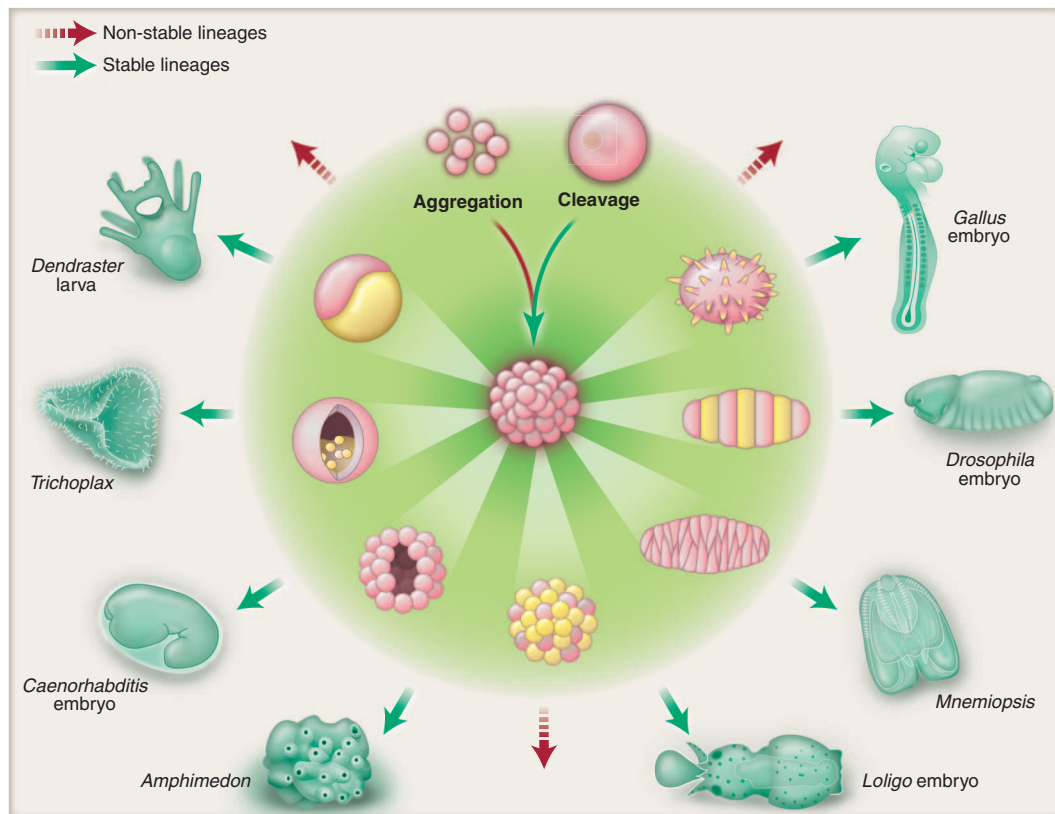


Fig. 1. A core set of physico-genetic modules underlies the morphological evolution of animals. Multicellular entities (center image) were formed by the aggregation of unicellular organisms (red curved arrow) or the cleavage of enlarged cells ["proto-eggs" (26) or eventually fertilized eggs] (green curved arrow). The green inner circle shows morphological motifs generated by some of the key DPMs: physical forces and effects relevant to the multicellular scale, mobilized by certain ancient single-cell gene products and pathways. Emergent motifs include (clockwise from top of inner circle) appendages, segments, elongated bodies and primordia, coexisting alternative cell types, interior cavities, dispersed cells, and multiple layers. Genetically uniform clusters produced stable lineages (straight green arrows), whereas chimeric clusters did not (broken red arrows). Contemporary organisms containing some or all of these motifs are shown in the outer circle. Clockwise from top right: vertebrate (*Gallus*) embryo, arthropod (*Drosophila*) embryo, ctenophore (*Mnemiopsis*), cephalopod (*Loligo*) embryo, demosponge (*Amphimedon*), nematode (*Caenorhabditis*) embryo, placozoan (*Trichoplax*), and echinoderm (*Dendroaster*) larva.

chemically and mechanically active soft materials. However, just as nonliving materials do not equally engage every physical effect, not every DPM appears in each animal lineage, because the relevant genes are not universally present throughout the metazoan phyla. The fundamental DPM is adhesion (mediated mainly by cadherins), which would have generated proto-metazoan clusters (Fig. 2). Such clusters, with appropriate DPM-enabling genes, could have exhibited more-complex body plans (Fig. 1, curved red arrow), but, as noted above, would have lost out to lineages arising by cleavage (Fig. 1, straight green arrows). The formation of non-intermixed layers, as in placozoans (Fig. 1), depended on differential interfacial tension (mediated by cadherins in conjunction with cytoskeletal mechanics) and apicobasal cell polarity (mediated by the canonical Wnt pathway). Lateral inhibition [mediated by the Notch pathway, absent in *Trichoplax* (27)] and a viscous, generalized (i.e., not epithelial or mesenchymal) extracellular matrix allowed the coexistence and rearrangement of contiguous intertransforming cells, as in sponges (Fig. 1). Planar polarity (mediated by the noncanonical Wnt pathway) and a basal lamina-type extracellular matrix [both absent in genetically characterized sponges and placozoans (27–29)] enabled the formation of elongated bodies and epithelial appendages and ridges, as in ctenophores (Fig. 1) and cndarians. An interstitial extracellular matrix allowed for epithelial-mesenchymal transformation and interactions, and triploblasty (i.e., three-layered structures). Extracellular matrices with distinctive physical properties (e.g., chitin versus collagen) and heterochrony in the developmental implementation of various shared DPMs led to disparate body plans among the triploblasts (Fig. 2), including

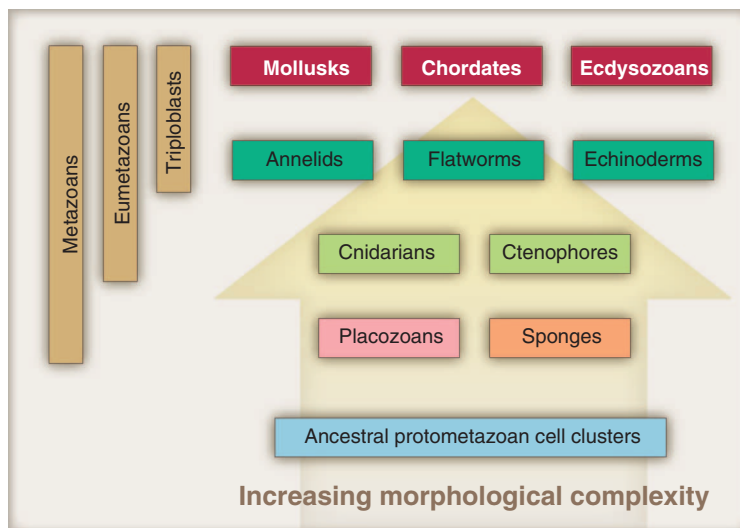


Fig. 2. The increasing complexity of animal body plans during evolution depended on the mobilization of new DPMs. The lines of descent of the various morphotypes are uncertain because of the possibility of gene loss and lateral transfer.

vertebrates, arthropods, nematodes, mollusks, and echinoderms (Fig. 1).

The idea that physics acted on early multicellular forms to define in broad strokes the patterns of development resolves several seemingly paradoxical aspects of the evolution of the animal phyla. These include the rapid emergence (in two episodes of approximately 20 million years each) of nearly all of the metazoan body plans during the late Ediacaran–early Cambrian periods (5, 30); the use of the same genetic tool kit to mediate similar morphogenetic processes in all animal phyla, however disparate (21); the recurrent appearance of a limited set of morphological motifs in all animal body plans and organ forms (20, 22); and the relative insensitivity of phylum-associated morphological signatures to variations at stages of development before the multicellular one, when DPMs come into play (26).

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10.1126/science.1222003

Genome Sequencing Identifies a Basis for Everolimus Sensitivity

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A long-standing problem in oncology is the variability of treatment response observed in early stage clinical trials. Drugs that fail to induce disease regression in most patients or prolong median progression-free survival (PFS) are deemed inactive and often abandoned, even when the drug exhibits profound activity in a small number of patients. We hypothesized that sequencing the tumor genomes of such “outlier” patients might identify unique somatic alterations that are the basis of their drug response, information that could in turn inform future clinical development.

We studied the tumor genome of a patient with metastatic bladder cancer who achieved a durable (>2 years) and ongoing complete response to everolimus, a drug targeting the mTORC1 (mammalian target of rapamycin) complex (Fig. 1A). The patient was enrolled in a phase II trial (ClinicalTrials.gov NCT00805 129) that failed to achieve its PFS end point. Whole-genome sequencing of DNA derived from the primary tumor and blood (1) iden-

tified 17,136 somatic missense mutations and small insertions and deletions (mutation rate of 6.21 per million bases). Of these, 140 were non-synonymous mutations within protein-coding or noncoding RNA regions of the genome. Structurally, this tumor genome was intact, lacking significant copy number alterations or functional translocations (Fig. 1B). Among confirmed coding mutations were (i) a two-base-pair deletion in the *TSC1* (tuberous sclerosis complex 1) gene, resulting in a frameshift truncation (c.1907_1908del, p.Glu636fs), and (ii) a nonsense mutation in the *NF2* (neurofibromatosis type 2) gene, creating a premature stop codon (c.863C>G, p.Ser288*). These loss-of-function mutations were noteworthy (table S1) because alterations in these genes have been associated with mTORC1 dependence in preclinical models (2). Sequencing of both genes in a second cohort of 96 high-grade bladder cancers identified five additional somatic *TSC1* mutations, whereas no additional *NF2* mutations were detected (fig. S1). Although the NF2 muta-

tion was uncommon in bladder cancers, knock-down of NF2 expression in TSC1-null bladder cancer cells was associated with enhanced sensitivity to mTORC1 inhibition (fig. S1).

Because *TSC1* is mutated in a subset of bladder cancers (3), we explored whether *TSC1* mutation is a biomarker of clinical benefit from everolimus therapy in this disease. We thus analyzed 13 additional bladder cancer patients treated with everolimus in the same trial with a targeted deep sequencing assay designed to interrogate the coding exons of ~200 genes commonly mutated in human cancers [(1) and fig. S1]. This analysis revealed three additional tumors harboring nonsense mutations in *TSC1*, including two patients who had minor responses to everolimus (Fig. 1C; 17 and 24% tumor regression). A fourth patient with 7% tumor regression had a somatic missense *TSC1* variant of unknown functional consequence. In contrast, tumors from eight of the nine patients showing disease progression were *TSC1* wild type. Patients with *TSC1*-mutant tumors remained on everolimus longer than those with wild-type tumors (7.7 versus 2.0 months, $P = 0.004$) with a significant improvement in time to recurrence (4.1 versus 1.8 months; hazard ratio = 18.5, 95% confidence interval 2.1 to 162, $P = 0.001$).

These results suggest that mTORC1-directed therapies may be most effective in cancer patients whose tumors harbor *TSC1* somatic mutations (4) and demonstrate the feasibility of using whole-genome and capture-based sequencing methodologies in the clinical setting to identify previously unrecognized biomarkers of drug response in genetically heterogeneous solid tumors. Although single-patient anecdotes are often dismissed as failing to provide meaningful clinical evidence, this example illustrates the potential for such cases to inform future clinical development of drugs in molecularly defined populations.

References and Notes

- Materials and methods are available as supplementary materials on Science Online.
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Acknowledgments: D.F.B. is a paid consultant for Novartis, which markets everolimus. M.F.B. is a paid consultant for Foundation Medicine, a company specializing in personalized cancer medicine. The compressed binary Sequence Alignment/Map files (BAM) have been deposited in dbGaP (accession no. phs000535.v1.p1).

Supplementary Materials

www.sciencemag.org/cgi/content/full/science.1226344/DC1

Materials and Methods

Fig. S1

Table S1

References (5–21)

20 June 2012; accepted 13 August 2012

Published online 23 August 2012;

10.1126/science.1226344

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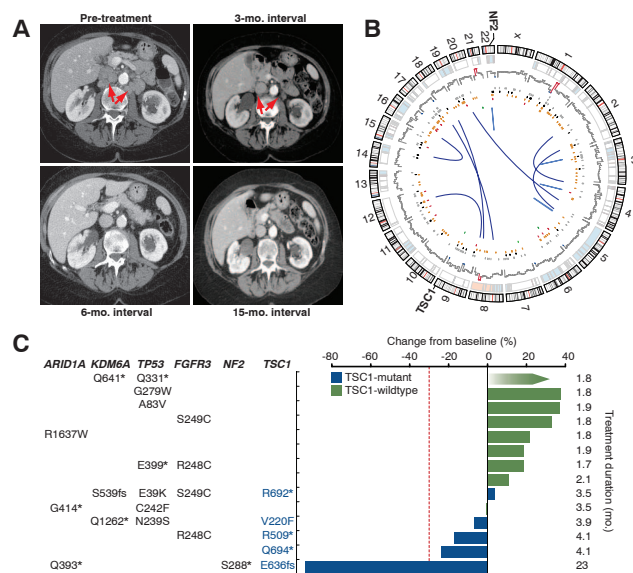


Fig. 1. (A) Computed tomography images of the index patient demonstrating complete resolution of metastatic disease (arrows). **(B)** Somatic abnormalities in the outlier responder's genome included (from outside to inside) copy number alterations; mutations at ~10-Mb resolution; regulatory, synonymous, missense, nonsense, nonstop, and frameshift insertion and deletion mutations (black, orange, red, green, and dark green); and intra- and interchromosomal rearrangements (light and dark blue). **(C)** Best overall response of 14 sequenced trial patients. Negative values indicate tumor shrinkage (red line, threshold for partial response). Gradient arrow, patient with rapid progression in bone.

A High-Coverage Genome Sequence from an Archaic Denisovan Individual

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We present a DNA library preparation method that has allowed us to reconstruct a high-coverage (30×) genome sequence of a Denisovan, an extinct relative of Neandertals. The quality of this genome allows a direct estimation of Denisovan heterozygosity indicating that genetic diversity in these archaic hominins was extremely low. It also allows tentative dating of the specimen on the basis of “missing evolution” in its genome, detailed measurements of Denisovan and Neandertal admixture into present-day human populations, and the generation of a near-complete catalog of genetic changes that swept to high frequency in modern humans since their divergence from Denisovans.

Draft genome sequences have been recovered from two archaic human groups, Neandertals (1) and Denisovans (2). Whereas Neandertals are defined by distinct morphological features and occur in the fossil record of Europe and western and central Asia from at least 230,000 until about 30,000 years ago (3), Denisovans are known only from a distal manual phalanx and two molars, all excavated at Denisova Cave in the Altai Mountains in southern Siberia (2, 4, 5). The draft nuclear genome sequence retrieved from the Denisovan phalanx revealed that Denisovans are a sister group to Neandertals (2), with the Denisovan nuclear genome sequence falling outside Neandertal genetic diversity, which suggests an inde-

pendent population history that differs from that of Neandertals. Also, whereas a genetic contribution from Neandertal to the present-day human gene pool is present in all populations outside Africa, a contribution from Denisovans is found exclusively in island Southeast Asia and Oceania (6).

Both published archaic genome sequences are of low coverage: 1.9-fold genomic coverage from the Denisovan phalanx and a total of 1.3-fold derived from three Croatian Neandertals. As a consequence, many positions in the genomes are affected by sequencing errors or nucleotide misincorporations caused by DNA damage. Previous attempts to generate a genome sequence of high coverage from an archaic human have been hampered by high levels of environmental contamination. The fraction of hominin endogenous DNA is commonly smaller than 1% and rarely approaches 5% (1, 7), which makes shotgun sequencing of the entire genome economically and logistically impractical. The only known exception is the Denisovan phalanx, which contains ~70% endogenous DNA. However, an extremely small fragment of this specimen is available to us, and the absolute number of endogenous molecules that could be recovered from the sample was too low to generate high genomic coverage.

A single-stranded library preparation method. DNA libraries for sequencing are normally prepared from double-stranded DNA. However, for ancient DNA the use of single-stranded DNA may be advantageous, as it will double its representation in the library. Furthermore, in a single-stranded DNA library, double-stranded molecules that carry modifications on one strand that prevent their incorporation into double-stranded DNA libraries could still be represented

by the unmodified strand. We therefore devised a single-stranded library preparation method wherein the ancient DNA is dephosphorylated, heat denatured, and ligated to a biotinylated adaptor oligonucleotide, which allows its immobilization on streptavidin-coated beads (Fig. 1). A primer hybridized to the adaptor is then used to copy the original strand with a DNA polymerase. Finally, a second adaptor is joined to the copied strand by blunt-end ligation, and the library molecules are released from the beads. The entire protocol is devoid of DNA purification steps, which inevitably cause loss of material.

We applied this method to aliquots of the two DNA extracts (as well as side fractions) that were previously generated from the 40 mg of bone that comprised the entire inner part of the phalanx (2, 8). Comparisons of these newly generated libraries with the two libraries generated in the previous study (2) show at least a 6-fold and 22-fold increase in the recovery of library molecules (8).

In addition to improved sequence yield, the single-strand library protocol reveals new aspects of DNA fragmentation and modification patterns (8). Because the ends of both DNA strands are left intact, it reveals that strand breakage occurs preferentially before and after guanine residues (fig. S6), which suggests that guanine nucleotides are frequently lost from ancient DNA, possibly as the result of depurination. It

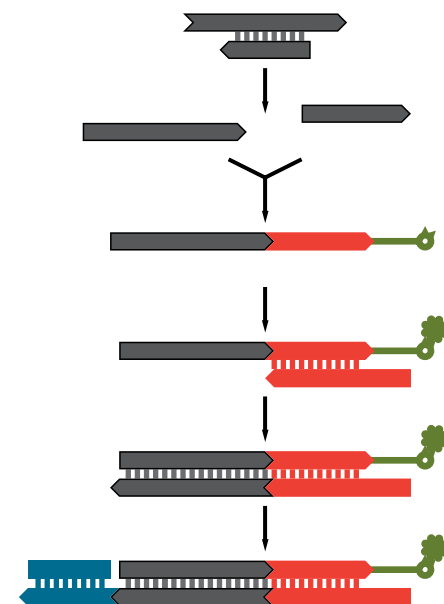


Fig. 1. For single-stranded library preparation, ancient DNA molecules are dephosphorylated and heat-denatured. Biotinylated adaptor oligonucleotides are ligated to the 3' ends of the molecules, which are immobilized on streptavidin-coated beads and copied by extension of a primer hybridized to the adaptor. One strand of a double-stranded adaptor is then ligated to the newly synthesized strand. Finally, the beads are destroyed by heat to release the library molecules (not shown).

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also reveals that deamination of cytosine residues occurs with almost equal frequencies at both ends of the ancient DNA molecules. Because deamination is hypothesized to be frequent in single-stranded DNA overhangs (9, 10), this suggests that 5' and 3' overhangs occur at similar lengths and frequencies in ancient DNA.

Genome sequencing. We sequenced these libraries from both ends using Illumina's Genome Analyzer IIX and included reads for two indexes (11), which were added in the clean room to exclude the possibility of downstream contamination with modern DNA libraries (1). Sequences longer than 35 base pairs (bp) were aligned to the human reference genome (GRCh37/1000 Genome project release) and the chimpanzee genome (CGSC 2.1/panTro2) with the Burrows-Wheeler Aligner (12). After removal of polymerase chain reaction duplicates, genotypes were called with the Genome Analysis Toolkit (8, 13). The three Denisovan libraries yielded 82.2 gigabases of nonduplicated sequence aligned to the human genome (8). Together with previous data (2), this provides about 31-fold coverage of the ~1.86 gigabases of the human autosomal genome to which short sequences can be confidently mapped (8). We also sequenced the genomes of 11 present-day individuals: a San, Mbuti, Mandenka, Yoruba, and Dinka from Africa; a French and Sardinian from Europe; a Han, Dai, and Papuan from Asia; and a Karitiana from South America. DNA from these individuals was bar-coded, pooled, and sequenced to ~24- to 33-fold genomic coverage (8). Because the samples were pooled, sequencing errors are the same across samples and are not expected to bias inferences about population relationships.

Genome quality. We used three independent measures to estimate human contamination in the Denisovan genome sequence (8). First, on the basis of a ~4100-fold coverage of the Denisovan mitochondrial (mt) genome, we estimate that 0.35% [95% confidence interval (C.I.) 0.33 to 0.36%] of fragments that overlap positions where the Denisovan mtDNA differs from most present-day humans show the modern human variant.

Second, because the Denisovan phalanx comes from a female (2), we infer male human DNA contamination to be 0.07% (C.I. 0.05 to 0.09%) from alignments to the Y chromosome. Third, a maximum-likelihood quantification of autosomal contamination gives an estimate of 0.22% (C.I. 0.22 to 0.23%). We conclude that less than 0.5% of the hominin sequences determined are extraneous to the bone (i.e., contamination from present-day humans).

Coverage of the genome is fairly uniform with 99.93% of the "mappable" positions covered by at least one, 99.43% by at least 10, and 92.93% by at least 20 independent DNA sequences (8). High-quality genotypes (genotype quality ≥ 40) could be determined for 97.64% of the positions. Whereas coverage in libraries prepared from ancient samples with previous methods is biased toward GC-rich sequences (14), the coverage of the libraries prepared with the single-stranded method from the Denisovan individual is similar to that of the 11 present-day human genomes (prepared from double-stranded DNA), in that coverage is positively correlated with AT-content (fig. S12).

To estimate average per-base error rates in Denisovan sequence reads, we counted differences between the sequenced DNA fragments and regions of the human genome that are highly conserved within primates [~5.6 million bases, (8)]. The error rate is 0.13% for the Denisovan genome, 0.17 to 0.19% for the genome sequences from the 11 present-day humans, and 1.2 to 1.7% for the two trios sequenced by the 1000 Genomes Pilot project (table S11). The lower Denisovan error rate per read is likely due to consensus-calling from duplicated reads representing the same DNA fragments and from overlap-merging of paired-end reads.

Molecular estimates of divergence and fossil age. We estimated the average DNA sequence divergence of all pair-wise combinations of the Denisovan genome and the 11 present-day humans as a fraction of the branch leading from the human-chimpanzee ancestor to present-day humans (Fig. 2) (8). If one assumes a human-chimpanzee average DNA sequence divergence of 6.5 million years ago (15), the Denisova-present-day human divergence is ~800,000 years, close to our previous estimate (2).

We next estimated the divergence of the archaic and modern human populations, which must be more recent than the DNA sequence divergence. To do this, we identified sites that are variable in a present-day west African individual, who is not affected by Denisovan or Neandertal gene flow, and counted how often the Denisovan and Neandertal genomes carry derived alleles not present in chimpanzee (1). From this, we estimate the population divergence between Denisovans and present-day humans to be 170,000 to 700,000 years (8). This is wider than our previous estimate (1), largely because it takes into account recent studies that broaden

the range of plausible estimates for human mutation rates and thus the human-chimpanzee divergence date.

When comparing the number of substitutions inferred to have occurred between the human-chimpanzee ancestor and the Denisovan and present-day human genomes, the number for the Denisovan genome is 1.16% lower (1.13 to 1.27%) (Fig. 2) (8). This presumably reflects the age of the Denisovan bone, which had less time to accumulate changes than present-day humans. Assuming 6.5 million years of sequence divergence between humans and chimpanzees, the shortening of the Denisovan branch allows the bone to be tentatively dated to between 74,000 and 82,000 years before present, in general agreement with the archaeological dates (2). However, we caution that multiple sources of error may affect this estimate (8). For example, the numbers of substitutions inferred to have occurred to the present-day human sequences vary by up to one-fifth of the reduction estimated for the Denisovan bone. Nevertheless, the results suggest that in the future it will be possible to determine dates of fossils based on genome sequences.

Denisovan and Neandertal gene flow. To visualize the relationship between Denisova and the 11 present-day humans, we used TreeMix, which simultaneously infers a tree of relationships and "migration events" (16) (Fig. 3). This method estimates that 6.0% of the genomes of present-day Papuans derive from Denisovans (8). This procedure does not provide a perfect fit to the data, for example, it does not model Neandertal admixture. An alternative method that incorporates Neandertal admixture yields an estimate of $3.0 \pm 0.8\%$ (8). This agrees with our previous finding that Denisovans have contributed to the genomes of present-day Melanesians, Australian aborigines, and other Southeast Asian islanders (2, 6).

We tested whether Denisovans share more derived alleles with any of the 11 present-day humans (8). To increase the power to detect gene flow, we used a new approach, "enhanced" *D*-statistics, which restricts the analysis to alleles that are not present in 35 African genomes and are thus more likely to come from archaic humans. This confirms that Denisovans share more alleles with Papuans than with mainland Eurasians (Fig. 4A and table S24). However, in contrast to a recent study proposing more allele-sharing between Denisovans and populations from southern China, such as the Dai, than with populations from northern China, such as the Han (17), we find less Denisovan allele-sharing with the Dai than with the Han (although nonsignificantly so, $Z = -0.9$) (Fig. 4B and table S25). Further analysis shows that if Denisovans contributed any DNA to the Dai, it represents less than 0.1% of their genomes today (table S26).

It is interesting that we find that Denisovans share more alleles with the three populations from eastern Asia and South America (Dai, Han, and Karitiana) than with the two European

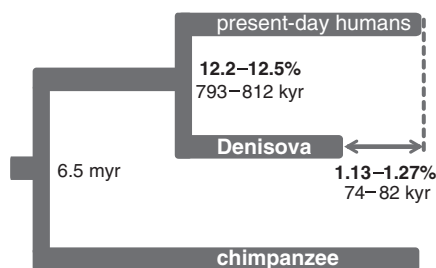


Fig. 2. Average sequence divergence and branch length differences between the Denisovan genome and 11 present-day humans represented as a tree. Divergence is reported as fraction of the branch leading from human to the common ancestor with chimpanzee, and in years, if one assumes a human-chimpanzee divergence of 6.5 million years ago.

populations (French and Sardinian) ($Z = 5.3$). However, this does not appear to be due to Denisovan gene flow into the ancestors of present-day Asians, because the excess archaic material is more closely related to Neandertals than to Denisovans (table S27). We estimate that the proportion of Neandertal ancestry in Europe is 24% less than in eastern Asia and South America (95% C.I. 12 to 36%). One possible explanation is that there were at least two independent Neandertal gene flow events into modern humans (18). An alternative explanation is a single Neandertal gene flow event followed by dilution of the Neandertal proportion in the ancestors of

Europeans due to later migration out of Africa. However, this would require about 24% of the present-day European gene pool to be derived from African migrations subsequent to the Neandertal admixture. Notably, Papuans share more alleles with the Denisovan genome on the autosomes than on the X chromosome ($P = 0.01$ by a two-sided test) (table S28). One possible explanation for this finding is that the gene flow into Papuan ancestors involved primarily Denisovan males. Another explanation is population substructure combined with predominantly female migration among the ancestors of modern humans as they

encountered Denisovans (which would have diluted the Denisovan component on chromosome X) (19). A third possibility is natural selection against hybrid incompatibility alleles, which are known to be concentrated on chromosome X (20). We note that some autosomes (e.g., chromosome 11) also have less Denisovan ancestry (table S30), which suggests that factors such as hybrid incompatibility may be at play.

Denisovan genetic diversity. The high quality of the Denisovan genome allowed us to measure its heterozygosity, i.e., the fraction of nucleotide sites that are different between a person's maternal and paternal genomes (Fig. 5A). Several methods indicate that the Denisovan heterozygosity is about 0.022% (8). This is ~20% of the heterozygosity seen in the Africans, ~26 to 33% of that in the Eurasians, and 36% of that in the Karitiana, a South American population with extremely low heterozygosity (21). Because we find no evidence for unusually long stretches of homozygosity in the Denisovan genome (8), this is not due to inbreeding among the immediate ancestors of the Denisovan individual. We thus conclude that the genetic diversity of the population to which the Denisovan individual belonged was very low compared with that of present-day humans.

To estimate how Denisovan and modern human population sizes have changed over time, we applied a Markovian coalescent model (22) to all genomes analyzed. This shows that present-day human genomes share similar population-size changes, in particular a more than twofold increase in size before 125,000 to 250,000 years ago (depending on the mutation rates assumed) (23) (Fig. 5B). Denisovans, in contrast, show a drastic decline in size at the time when the modern human population began to expand.

A prediction from a small ancestral Denisovan population size is that natural selection would

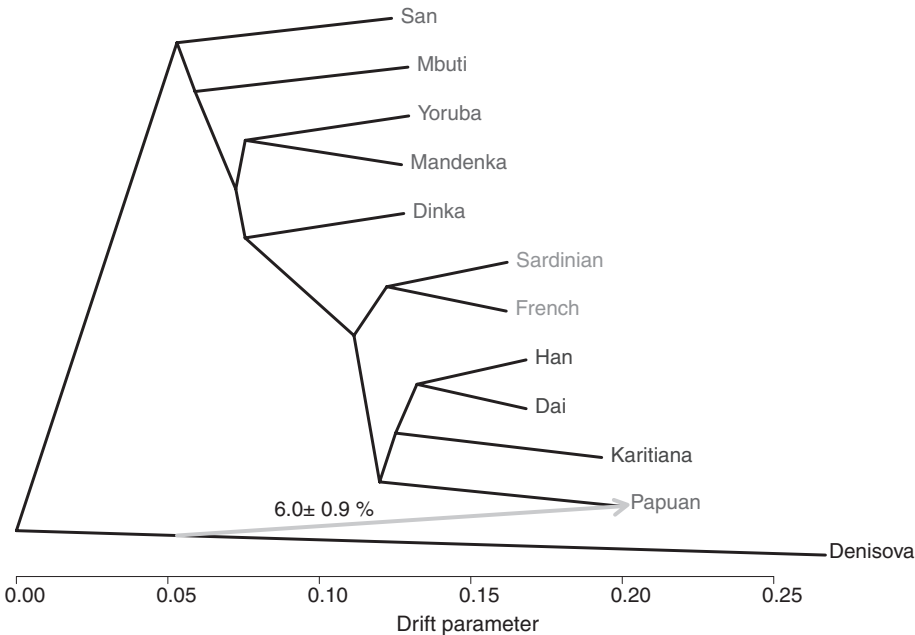
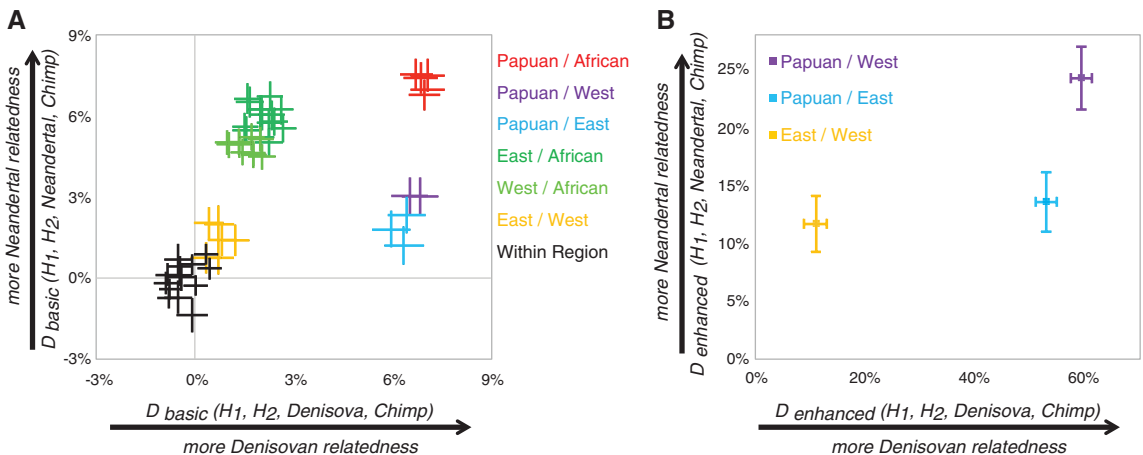


Fig. 3. Maximum likelihood tree relating the Denisovan genome and the genomes of 11 present-day humans, allowing one migration event (shown as a gray arrow).

Fig. 4. (A) Sharing of derived alleles among present-day humans, Denisovans, and Neandertals. We compare all possible pairs of 11 present-day humans (H_1, H_2) in their D -statistics, which measure the rate at which they share derived alleles with Denisovans (x axis) and Neandertals (y axis). Each point reports ± 1 standard error bars from a block jackknife. D -statistics are color-coded by geographic region ("East" and "West" refer to Eurasia). Note that the D -statistic is not the same as the mixture proportion, as it is also affected, for example, by the amount of genetic drift that is shared between the samples. **(B)** Sharing of derived alleles that are absent in Africans among present-day humans, Denisovans, and Neandertals. We enhance the power of the D -statistics by restricting the analysis to sites where 35 sub-Saharan Africans



have the ancestral allele and by pooling modern humans by region (bars again give one standard error). Eastern non-African populations have significantly more archaic ancestry than Western populations ($Z = 5.3$ and $Z = 4.8$ for the tests based on the Denisovan and Neandertal D -statistics, respectively).

be less effective in weeding out slightly deleterious mutations. We therefore estimated the ratio of nonsynonymous substitutions that are predicted to have an effect on protein function to synonymous substitutions (those that do not change amino acids) in the genomes analyzed and found it to be, in Denisovans, on average 1.5 to 2.5 times that in present-day humans, depending on the class of sites and populations to which Denisovans are compared (Fig. 5C) (8). This is consistent with Denisovans having a smaller population size than modern humans, resulting in less-efficient removal of a deleterious mutation.

Denisovan genomic features. Because almost no phenotypic information exists about Denisovans, it is of some interest that, in agreement with a previous study (24), the Denisovan individual carried alleles that in present-day humans are associated with dark skin, brown hair, and brown eyes (table S58) (8). We also identified nucleotide changes specific to this Denisovan individual and not shared with any present-day human (8). However, since we have access to only a single Denisovan individual, we expect that only a subset of these would have been shared among all Denisovans.

Of more relevance may be examination of aspects of the Denisovan karyotype. The great apes have 24 pairs of chromosomes whereas hu-

mans have 23. This difference is caused by a fusion of two acrocentric chromosomes that formed the metacentric human chromosome 2 (25) and resulted in the unique head-to-head joining of the telomeric hexameric repeat GGGGTT. A difference in karyotype would likely have reduced the fertility of any offspring of Denisovans and modern humans. We searched all DNA fragments sequenced from the Denisovan individual and identified 12 fragments containing joined repeats. By contrast, reads from several chimpanzees and bonobos failed to yield any such fragments (8). We conclude that Denisovans and modern humans (and presumably Neandertals) shared the fused chromosome 2.

Features unique to modern humans. Genome sequences of archaic human genomes allow the identification of derived genomic features that became fixed or nearly fixed in modern humans after the divergence from their archaic relatives. The previous Denisovan and Neandertal genomes (1, 2) allowed less than half of all such features to be assessed with confidence. The current Denisovan genome enables us to generate an essentially complete catalog of recent changes in the human genome accessible with short-read technology (26). In total, we identified 111,812 single-nucleotide changes (SNCs) and 9499 insertions and deletions where modern humans are fixed for the derived state, whereas the Denisovan

individual carried the ancestral, i.e., ape-like, variant (8). This is a relatively small number. We identified 260 human-specific SNCs that cause fixed amino acid substitutions in well-defined human genes, 72 fixed SNCs that affect splice sites, and 35 SNCs that affect key positions in well-defined motifs within regulatory regions.

One way to identify changes that may have functional consequences is to focus on sites that are highly conserved among primates and that have changed on the modern human lineage after separation from Denisovan ancestors. We note that, among the 23 most conserved positions affected by amino acid changes (primate conservation score of ≥ 0.95), eight affect genes that are associated with brain function or nervous system development (*NOVA1*, *SLITRK1*, *KATNA1*, *LUZP1*, *ARHGAP32*, *ADSL*, *HTR2B*, and *CNTNAP2*). Four of these are involved in axonal and dendritic growth (*SLITRK1* and *KATNA1*) and synaptic transmission (*ARHGAP32* and *HTR2B*), and two have been implicated in autism (*ADSL* and *CNTNAP2*). *CNTNAP2* is also associated with susceptibility to language disorders (27) and is particularly noteworthy as it is one of the few genes known to be regulated by *FOXP2*, a transcription factor involved in language and speech development as well as synaptic plasticity (28). It is thus tempting to speculate that crucial aspects of synaptic transmission may have changed in modern humans.

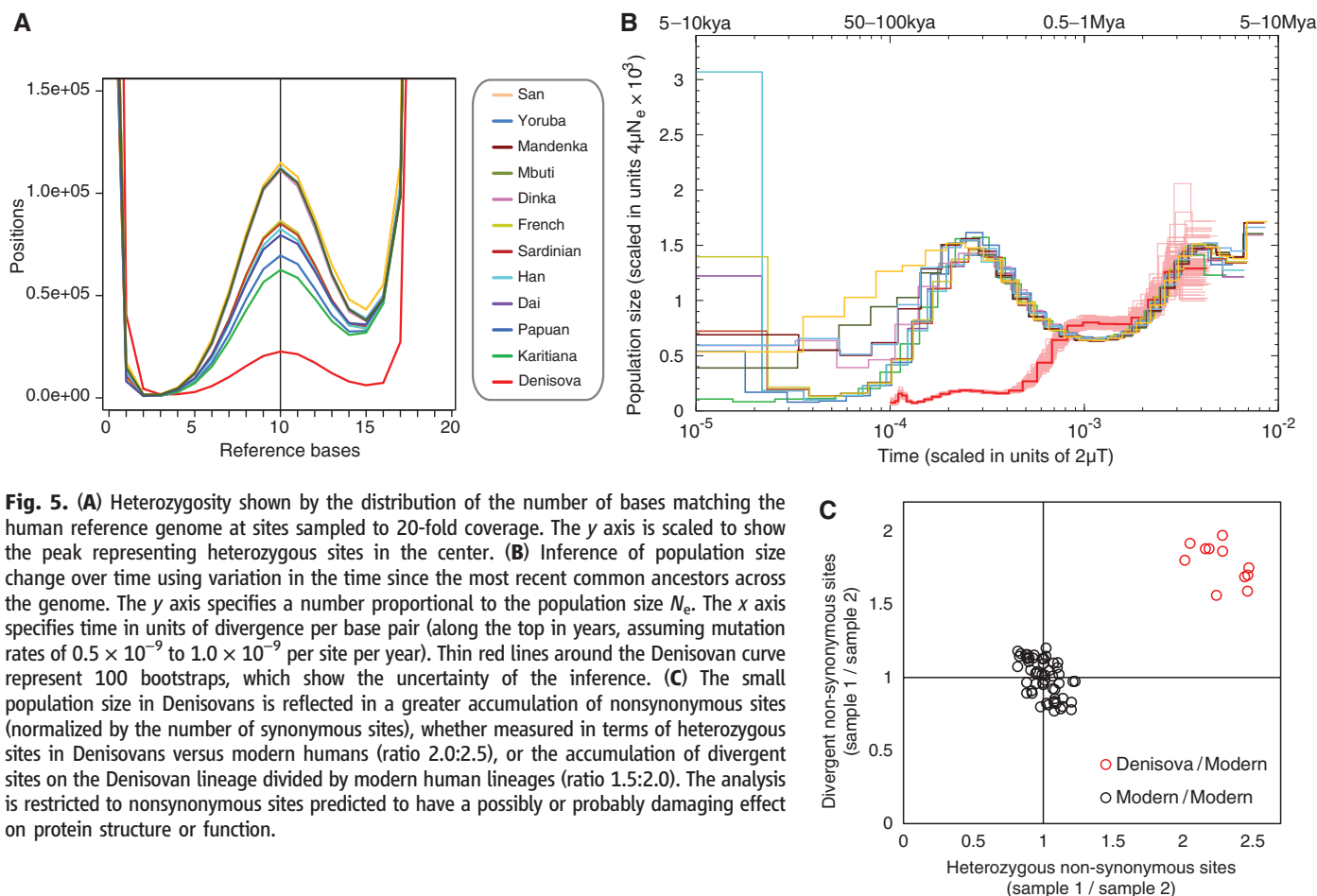


Fig. 5. (A) Heterozygosity shown by the distribution of the number of bases matching the human reference genome at sites sampled to 20-fold coverage. The y axis is scaled to show the peak representing heterozygous sites in the center. **(B)** Inference of population size change over time using variation in the time since the most recent common ancestors across the genome. The y axis specifies a number proportional to the population size N_e . The x axis specifies time in units of divergence per base pair (along the top in years, assuming mutation rates of 0.5×10^{-9} to 1.0×10^{-9} per site per year). Thin red lines around the Denisovan curve represent 100 bootstraps, which show the uncertainty of the inference. **(C)** The small population size in Denisovans is reflected in a greater accumulation of nonsynonymous sites (normalized by the number of synonymous sites), whether measured in terms of heterozygous sites in Denisovans versus modern humans (ratio 2.0:2.5), or the accumulation of divergent sites on the Denisovan lineage divided by modern human lineages (ratio 1.5:2.0). The analysis is restricted to nonsynonymous sites predicted to have a possibly or probably damaging effect on protein structure or function.

Our limited understanding of how genes relate to phenotypes makes it impossible to predict the functional consequences of these changes. However, diseases caused by mutations in genes offer clues as to which organ systems particular genes may affect. Of the 34 genes with clear associations with human diseases that carry fixed substitutions changing the encoded amino acids in present-day humans, four (*HPS5*, *GGCX*, *ERCC5*, and *ZMPSTE24*) affect the skin and six (*RP1L1*, *GGCX*, *FRMD7*, *ABCA4*, *VCAN*, and *CRYBB3*) affect the eye. Thus, particular aspects of the physiology of the skin and the eye may have changed recently in human history. Another fixed difference occurs in *EVC2*, which when mutated causes Ellis-van Creveld syndrome. Among other symptoms, this syndrome includes taurodontism, an enlargement of the dental pulp cavity and fusion of the roots, a trait that is common in teeth of Neandertals and other archaic humans. A Denisovan molar found in the cave has an enlarged pulp cavity but lacks fused roots (2). This suggests that the mutation in *EVC2*, perhaps in conjunction with mutations in other genes, has caused a change in dental morphology in modern humans.

We also examined duplicated regions larger than 9 kilobase pairs (kbp) in the Denisovan and present-day human genomes and found the majority of them to be shared (8). However, we find 10 regions that are expanded in all present-day humans but not in the Denisovan genome. Notably, one of these overlaps a segmental duplication associated with a pericentric inversion of chromosome 18. In contrast to humans, the Denisovan genome harbors only a partial duplication of this region, which suggests that a deletion occurred in the Denisovan lineage. However, we are unable to resolve if the pericentric inversion is indeed present in Denisovans.

Implications for archaic and modern human history. It is striking that genetic diversity among Denisovans was low although they were present in Siberia as well as presumably in Southeast Asia where they interacted with the ancestors of present-day Melanesians (6). Only future research can show how wide their geographic range was at any one time in their history. However, it is likely that they have expanded from a small population size with not enough time elapsing for genetic diversity to correspondingly increase. When technical improvements such as the one presented here will make it possible to sequence a Neandertal genome to a quality comparable to the Denisovan and modern genomes, it will be important to clarify whether the temporal trajectory of Neandertal effective population size matches that of the Denisovans. If that is the case, it is likely that the low Denisovan diversity reflects the expansion out of Africa of a population ancestral to both Denisovans and Neandertals, a possibility that seems compatible with the dates for population divergences and population size changes presented.

By providing a comprehensive catalog of features that became fixed in modern humans after

their separation from their closest archaic relatives, this work will eventually lead to a better understanding of the biological differences that existed between the groups. This should ultimately aid in determining how it was that modern humans came to expand dramatically in population size as well as cultural complexity while archaic humans eventually dwindled in numbers and became physically extinct.

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Acknowledgments: The Denisovan sequence reads are available from the European Nucleotide Archive (ENA) under study accession ERP001519. The present-day human sequence reads are available from the Short Read Archive (SRA) under accession SRA047577. Alignments and genotype calls for each of the sequenced individuals are available at www.eva.mpg.de/denisova/. In addition, the Denisovan sequence reads and alignments are available as a public data set via Amazon Web Services (AWS) at <http://aws.amazon.com/datasets/2357/> and as a track in the University of California, Santa Cruz genome browser. We thank D. Falush, P. Johnson, J. Krause, M. Lachmann, S. Sawyer, L. Vigilant and B. Viola for comments, help, and suggestions; A. Aximu, B. Höber, B. Höffner, A. Weihmann, T. Kratzer, and R. Roesch for expert technical assistance; R. Schultz for help with data management; and M. Schreiber for improvement of graphics. The Presidential Innovation Fund of the Max Planck Society made this project possible. D.R. and N.P. are grateful for support from NSF HOMINID grant no. 1032255 and NIH grant GM100233. J.G.S., F.J., and M.S. were supported by NIH grant R01-GM40282 to M.S. P.H.S. is supported by an HHMI International Student Fellowship. F.R. is supported by a German Academic Exchange Service (DAAD) study scholarship. E.E.E. is on the scientific advisory boards for Pacific Biosciences, Inc., SynapDx Corp, and DNAnexus, Inc.

Supplementary Materials

www.sciencemag.org/cgi/content/full/science.1224344/DC1
Materials and Methods
Figs. S1 to S38
Tables S1 to S58
References (29–196)

7 May 2012; accepted 14 August 2012
Published online 30 August 2012;
10.1126/science.1224344

Cilia at the Node of Mouse Embryos Sense Fluid Flow for Left-Right Determination via Pkd2

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Unidirectional fluid flow plays an essential role in the breaking of left-right (L-R) symmetry in mouse embryos, but it has remained unclear how the flow is sensed by the embryo. We report that the Ca²⁺ channel Polycystin-2 (Pkd2) is required specifically in the perinodal crown cells for sensing the nodal flow. Examination of mutant forms of Pkd2 shows that the ciliary localization of Pkd2 is essential for correct L-R patterning. Whereas *Kif3a* mutant embryos, which lack all cilia, failed to respond to an artificial flow, restoration of primary cilia in crown cells rescued the response to the flow. Our results thus suggest that nodal flow is sensed in a manner dependent on Pkd2 by the cilia of crown cells located at the edge of the node.

Most of the visceral organs in vertebrates exhibit left-right (L-R) asymmetry in their shape and/or position. The break-

ing of L-R symmetry in the embryos of many vertebrates is mediated by a unidirectional fluid flow in the ventral node (an embryonic cavity

at the midline filled with extra-embryonic fluid) or its equivalent structure (1, 2). The flow may transport a determinant molecule or provide mechanical force. However, it has remained unclear how the flow is sensed. We have now addressed this issue with the use of mice lacking Polycystin-2 (*Pkd2*, also known as TRPP2), a gene encoding a Ca^{2+} -permeable cation channel implicated in polycystic kidney disease in humans (3, 4).

Pkd2 required only in perinodal crown cells.

The *Pkd2*^{−/−} mouse was previously shown to lose *Nodal* expression in the lateral plate mesoderm (LPM) and to exhibit typical L-R patterning defects (5). *Pkd2* is expressed ubiquitously at the early somite stage of mouse embryos (fig. S1) (5); however, it has been unclear whether the function of *Pkd2* in L-R patterning is executed in the node, LPM, or other region of the embryo. We first examined whether the LPM of the *Pkd2*^{−/−} embryo is competent for *Nodal* signaling. When an expression vector for *Nodal* was introduced to a small region of the right LPM of the *Pkd2*^{−/−} embryo, expression of endogenous *Nodal* gene was induced in the entire region of the right LPM (fig. S2), suggesting that the LPM of the *Pkd2*^{−/−} embryo remains competent to express *Nodal*. To further localize the site of *Pkd2* action, we established three types of transgenic mice that express *Pkd2* specifically in the node and examined whether such transgenes were able to prevent the L-R defects of the *Pkd2*^{−/−} mouse (Fig. 1 and fig. S3). In one transgene, *Foxa2*^{2NNE-hsp-Pkd2-IRES-LacZ (Fig. 1A), *Pkd2* expression is driven by a node/notochord-specific enhancer of the mouse *Foxa2* gene. This transgene is expressed in the midline including the node (Fig. 1A) and restored left-sided expression of *Nodal* and *Pitx2* in the LPM of *Pkd2*^{−/−} embryos (6 rescued of 6 tested embryos for *Nodal* and 6 of 6 for *Pitx2*) (Fig. 1C).}

The ventral node contains two different types of ciliated cells: pit cells located in the central re-

gion of the node and crown cells located at the edge (fig. S3A) (1). The second *Pkd2* transgene studied, *NDE-hsp-Pkd2-IRES-LacZ* (Fig. 1B), is driven by the node-specific enhancer (NDE) of *Nodal*, which is active specifically in the crown cells. This transgene was able to rescue left-sided expression of *Nodal* and *Pitx2* in the LPM of *Pkd2*^{−/−} embryos (6 of 6 and 3 of 3, respectively) (Fig. 1C). Last, expression of *Pkd2* specifically in the pit cells with *dgFoxa2-hsp-Pkd2-IRES-LacZ* (fig. S3B) was unable to rescue the left-sided expression of *Nodal* and *Pitx2* (0 of 1 and 0 of 3 embryos, respectively) (fig. S3C). These results thus suggested that *Pkd2* is required exclusively in crown cells of the node for correct L-R determination.

***Cerl2* the major target of *Pkd2*-mediated signaling.** Initial breaking of L-R symmetry by leftward nodal flow is followed by asymmetric (R>L) expression of *Cerl2* in crown cells (6, 7). Given that *Pkd2* is required specifically in crown cells, we examined crown cell-specific markers in *Pkd2*^{−/−} embryos. *Gdf1*, which is bilaterally coexpressed in crown cells and is required for subsequent *Nodal* expression in left LPM (8, 9), was normally expressed in *Pkd2*^{−/−} embryos (fig. S4A). However, L-R asymmetric gene expression in crown cells was impaired in the mutant embryos. Expression of *Cerl2* in crown cells is greater on the right side in wild-type embryos (fig. S4A) (6) but was expressed equally on the two sides in *Pkd2*^{−/−} embryos (fig. S4A). *Nodal* expression, which shows a subtle asymmetry in the wild-type embryo (right expression lower than left) (10), was bilaterally equal in *Pkd2*^{−/−} embryos (fig. S4A). These results suggested that crown cells are correctly specified but lose asymmetric gene expression in the absence of *Pkd2*.

The higher expression of *Cerl2* on the right in crown cells is required for subsequent asymmetric expression of *Nodal* in LPM (6). To clarify the relation between *Pkd2* and *Cerl2*, we examined possible interaction between the two genes by analyzing *Pkd2*^{−/−};*Cerl2*^{−/−} double-mutant embryos (fig. S4B). *Nodal* expression in LPM was always absent in *Pkd2*^{−/−} embryos (5 of 5), whereas it was either left-sided (2 of 11), right-sided (3 of 11), bilateral (2 of 11), or absent (4 of 11) in *Pkd2*^{−/−};*Cerl2*^{−/−} embryos (fig. S4, B and C). The *Nodal* expression pattern in the double mutant was thus similar to that in *Cerl2*^{−/−} embryos (fig. S4, B and C), indicating that *Cerl2* is the major target of *Pkd2*-mediated signaling for L-R asymmetric patterning (fig. S4D).

***Pkd2* in crown cells required for sensing nodal flow.** Particle image velocimetry (PIV) revealed that nodal flow is maintained in the *Pkd2*^{−/−} mutant (Fig. 2A), suggesting that *Pkd2* might serve to sense a flow-derived signal (or signals) in crown cells. We tested this possibility with the use of a transcriptional enhancer that responds to nodal flow. This enhancer (ANE) is derived from the human *Lefty1* gene and exhibits asymmetric (L>R) activity in crown cells (Fig. 2B) (11). Furthermore, ANE was found to respond to artificial flow. The

relative L-R activity of ANE was thus reversed when a rightward flow was imposed on the wild-type embryo (Fig. 2, C and D). In *Pkd2*^{−/−} embryos, ANE activity was R=L (Fig. 2B), despite the fact that the mutant possesses morphologically normal cilia (fig. S5) and functional flow (Fig. 2A), suggesting that crown cells of *Pkd2*^{−/−} embryos fail to sense the flow.

Given that *Pkd2* encodes a Ca^{2+} -permeable ion channel, we examined Ca^{2+} signaling in crown cells by generating a transgenic mouse that expresses Ca^{2+} indicator GCaMP2, which exhibits highly cooperative Ca^{2+} binding over the physiological range of cellular free Ca^{2+} [dissociation constant (K_d) = 146 nM] (12), specifically in crown cells. Indeed, Ca^{2+} signaling was detected in crown cells, but it was present bilaterally and was retained in *Pkd2*^{−/−} embryos (fig. S6). Ca^{2+} asymmetry previously observed with exogenous dyes (13, 14) occurs in the endoderm, a different tissue around the node. To clarify the role of Ca^{2+} signaling, transgenic embryos harboring *ANE-lacZ* were incubated with various Ca^{2+} signaling blockers. GdCl₃ (an inhibitor of stretch-sensitive TRP channels), 2-ABP (an inhibitor of IP₃ receptor), and Thapsigargin (an inhibitor of Ca^{2+} -ATPase in ER) disrupted L>R asymmetry of ANE activity, whereas Ruthenium Red (a potent inhibitor of intracellular Ca^{2+} release by ryanodine receptors) did not (Fig. 3). Treatment with Thapsigargin did not significantly reduce the level of Ca^{2+} signaling within crown cells (fig. S7), likely because a single inhibitor would only affect a portion of Ca^{2+} signaling within a cell. Rotational movement of pit cell cilia and ciliary localization of *Pkd2* were maintained by treatment with GdCl₃ and Thapsigargin (fig. S8). These results suggest that Ca^{2+} signaling mediated by *Pkd2* and IP₃ receptor is essential for generating L-R asymmetry at the node (Fig. 3B).

Ciliary localization of *Pkd2* essential for correct L-R decision. *Pkd2* protein resides in the primary cilia of renal epithelial cells (15). In crown cells as well as pit cells of the node, endogenous *Pkd2* was also localized in the cilia (fig. S9A) (13). We also examined the subcellular localization of *Pkd2* with the use of a transgenic mouse that expresses a *Pkd2*::Venus fusion protein in crown cells (Fig. 4A). This fusion protein was shown to be functional by the observation that its expression in crown cells corrected the L-R defects of *Pkd2*^{−/−} embryos and was preferentially localized to the cilia of crown cells (Fig. 4A). Expression of *Pkd2*::Venus protein did not influence motility of cilia (movie S1). Live imaging of *Pkd2*::Venus-labeled cilia at the presomite stage showed that most of the cilia were immotile (fig. S10 and movie S2). The frequency of motile cilia gradually increased between the presomite stage and the three-somite stage (fig. S10). However, most (~90%) crown cell cilia were immotile, whereas ~60% of pit cell cilia are already motile at the presomite stage (fig. S10), the stage at which *Cerl2* expression in crown cells begins to exhibit L-R asymmetry (11).

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Ciliary localization of Pkd2 may depend on its interaction with Pkd111, another ciliary protein whose deficiency in mice and zebrafish results in L-R defects similar to those of the *Pkd2*^{-/-} mutant (16, 17). To test whether Pkd2 may function in cilia of crown cells, various mutant forms of Pkd2 were examined for their abilities to localize to crown cell cilia and to correct the L-R defects of *Pkd2*^{-/-} embryos (Fig. 4, B to E; Table 1; and figs. S9 and S11). Although a missense mutation of Pkd2 (E442G) induces L-R defects identical to those of *Pkd111* mutant mouse (18), a Pkd2(E442G)::Venus fusion protein was unable to localize to the cilia of crown cells (Fig. 4, B and C) and cilia of cultured LLC-PK1 cells (fig. S12). Pkd2(E442G) can interact with Pkd111 (fig. S13), suggesting that ciliary localization of Pkd2 requires Pkd111-independent mechanisms. Pkd2(R6G), a missense mutant unable to localize to primary cilia in cultured LLC-PK1 cells (19), was found to localize to the cilia of crown cells (fig. S9B) and rescued L-R patterning in the

Pkd2^{-/-} mutant (fig. S11). Pkd2(Δ5–73), which lacks the NH₂-terminal region (residues 5 to 73) including the R₆VxP motif (19), was also able to localize to the cilium when expressed in crown cells (fig. S9C). Pkd2(R6G-G819X), which harbors the R6G missense mutation and lacks the COOH-terminal region, was unable to localize to the crown cell cilium (fig. S9D) or to correct the L-R defects of the *Pkd2*^{-/-} mutant (fig. S11). Similarly, Pkd2(D509V)—a missense mutant associated with polycystic kidney disease in humans (20)—failed both to localize to cilia of crown cells (Fig. 4B) and to normalize the *Pkd2*^{-/-} phenotype (fig. S11). Two mutants that were unable to localize to crown cell cilia (E442G and R6G-G819X) were examined for their channel activity (Fig. 4D). Although Pkd2(R6G-G819X) lost channel activity, Pkd2(E442G) retained it (Fig. 4D). Our findings that Pkd2(E442G) retains channel activity yet is unable to localize to crown cell cilia (Fig. 4, B and C) indicate that ciliary localization of Pkd2 in crown cell is essential for correct L-R decision.

Crown cell cilia as sensors of nodal flow. Last, we examined directly whether cilia of the crown cells function as sensors of nodal flow with the use of an IFT (intraflagellar transport) mouse mutant. *Kif3a* and *Kif3b* are expressed ubiquitously in mouse embryo and encode motor proteins that are required for formation of cilia (21–23). *Kif3a*^{-/-} mutant embryos thus lack all node cilia, including those of both pit and crown cells, and exhibit L-R defects (22, 23). A transgene that confers *Kif3a* expression specifically in crown cells (*NDE-hsp-Kif3a-IRES-LacZ*) restored cilia formation exclusively in these cells (Fig. 5A). Although *Nodal* expression in LPM was bilateral (L=R) in *Kif3a*^{-/-} embryos (8/8), restoration of cilia formation in crown cells by the *NDE-Kif3a* transgene rescued left-sided *Nodal* expression in LPM in *Kif3a*^{-/-} embryos (3 of 3) (Fig. 5, B and C). PIV analysis revealed a weak leftward flow as well as vortical flow in the node of such embryos (fig. S14), likely because the number of motile cilia among crown cells is much smaller than that of immotile cilia

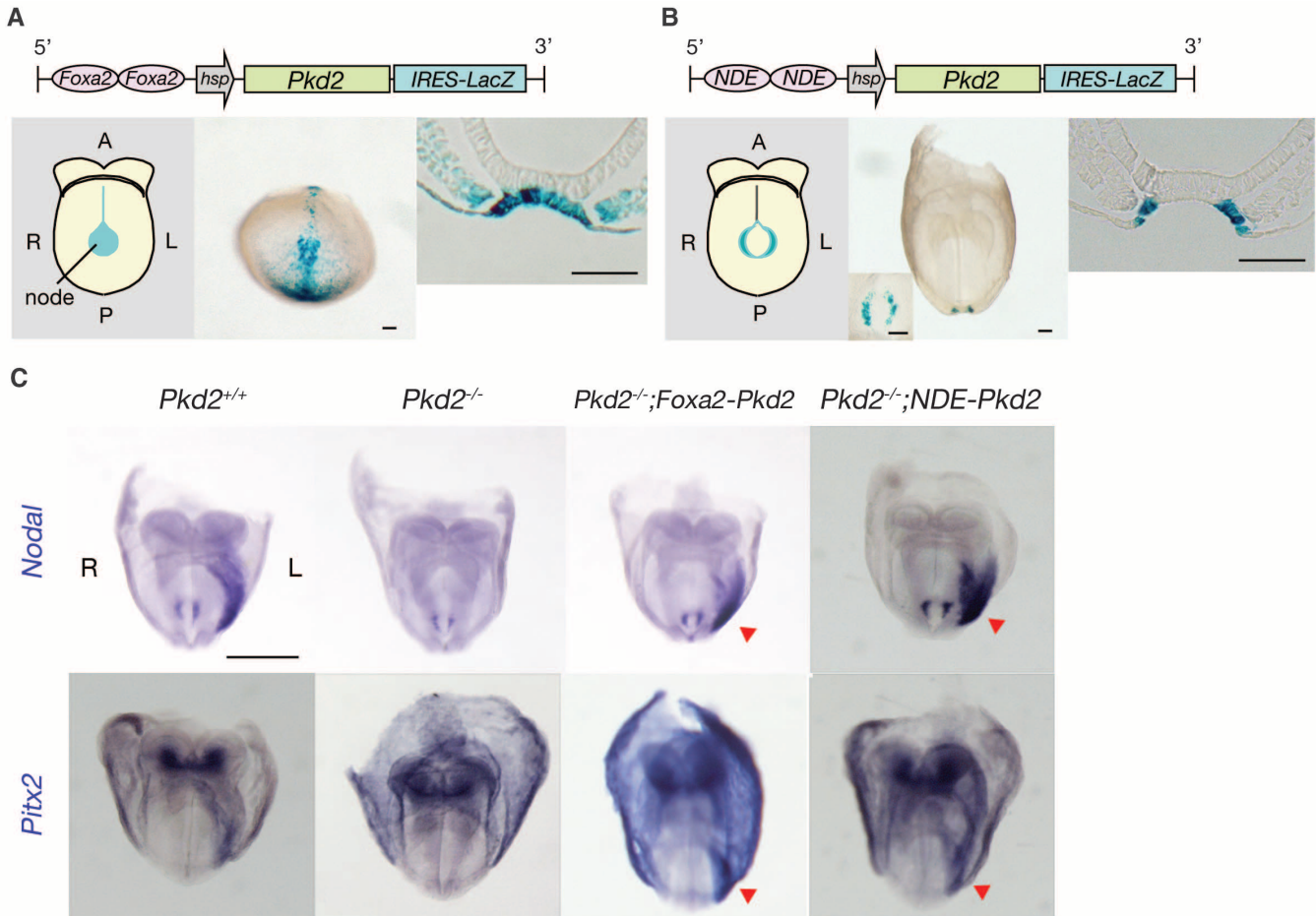


Fig. 1. Specific requirement for Pkd2 in crown cells of the node for correct L-R patterning. (A and B) Schematic representation of two types of node-specific *Pkd2* transgene are shown on the top. Their expression patterns were examined by staining of transgenic embryos at embryonic day 8.0 (E8.0) with X-gal. Schematic (left) and actual (middle) views of whole embryos, and transverse sections of the node (right) are shown. Middle, inset of (B) shows the node from ventral view at higher magnification.

Expression of the NDE-driven transgene is highly specific to crown cells of the node (B). A, anterior; P, posterior; L, left; R, right. Scale bars, 50 μ m. (C) Whole-mount in situ hybridization analysis of the expression of *Nodal* and *Pitx2* in E8.0 embryos of the indicated genotypes. Left-sided expression of *Nodal* and *Pitx2* in LPM is lost in *Pkd2*^{-/-} embryos but is restored (red arrowheads) in *Pkd2*^{-/-};Foxa2-Pkd2 and *Pkd2*^{-/-};NDE-Pkd2 embryos. Scale bar, 500 μ m.

(fig. S10). This result is consistent with our recent finding that as few as two rotating cilia are sufficient for symmetry breaking (24).

We then examined whether the embryos with cilia only in the crown cells are able to respond to artificial fluid flow. The *Kif3a*^{-/-}; *NDE-Kif3a* em-

bryos, as well as control *Kif3a*^{-/-} embryos, were thus subjected to rightward artificial flow (Fig. 5B), and L-R patterning as revealed by *Pitx2* expression

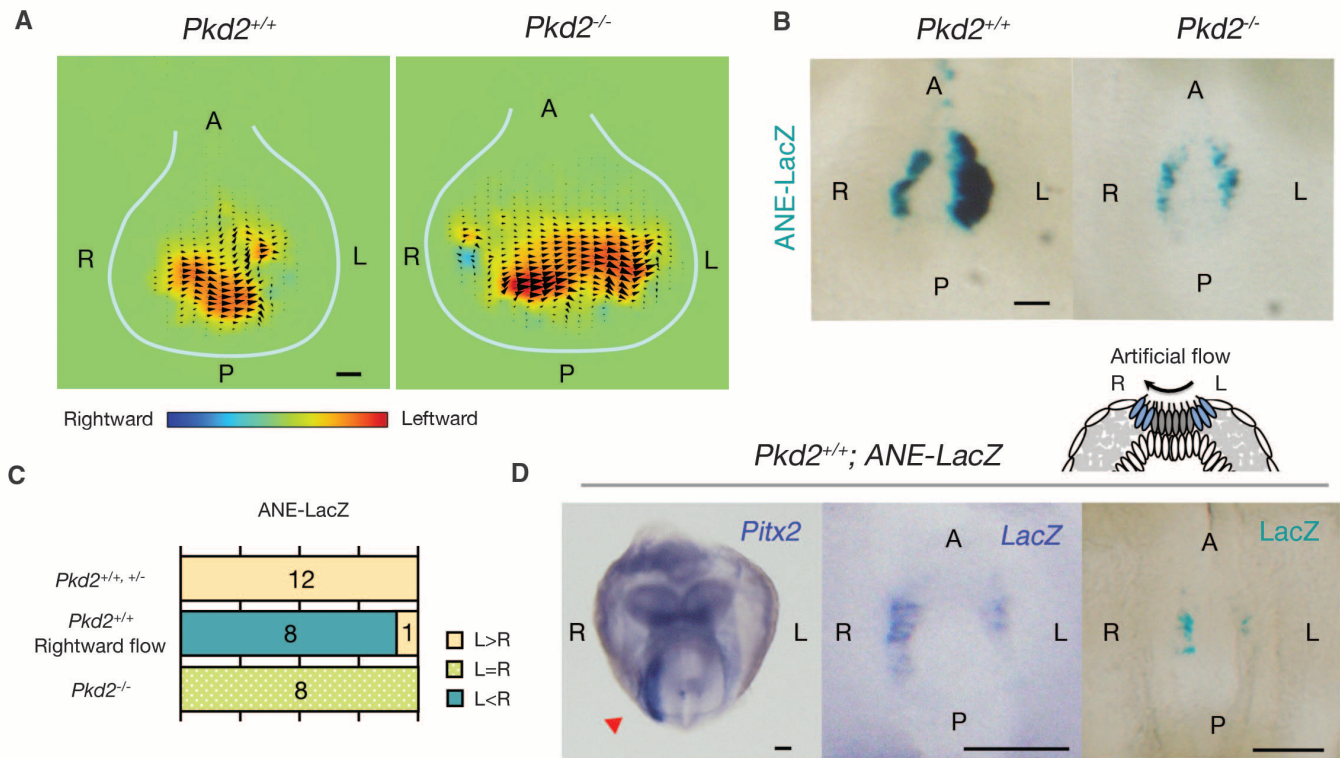
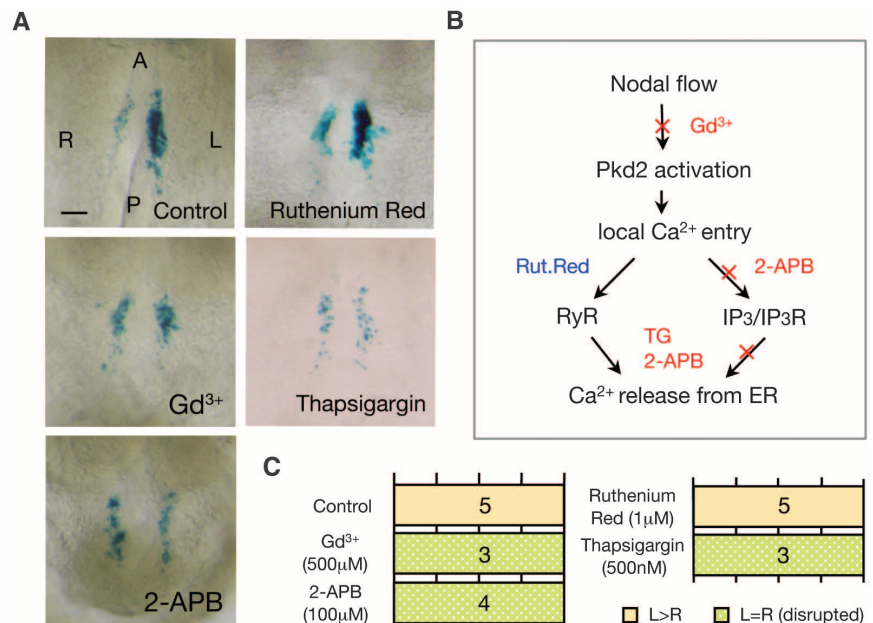


Fig. 2. *Pkd2* is necessary for sensing of nodal flow. (A) Nodal flows in wild-type and *Pkd2*^{-/-} embryos were examined by means of PIV analysis at E8.0. Flow is maintained in the *Pkd2*^{-/-} embryo. Each arrowhead denotes the direction and speed of the flow at the indicated position. The color scale indicates the direction and magnitude of the flow velocity (leftward in yellow and red, rightward in blue). White lines indicate the outlines of the node. Scale bar, 10 μ m. (B) E8.0 *Pkd2*^{+/+} and *Pkd2*^{-/-} embryos harboring the *ANE-LacZ* transgene were stained with X-gal. Scale bar, 50 μ m. (C) The L-R patterns of

ANE activities in embryos of the indicated genotypes either in situ or under conditions of artificial rightward flow in vitro are summarized. The numbers of embryos showing each pattern are indicated. (D) *Pkd2*^{+/+} embryos with *ANE-LacZ* were cultured under the influence of a rightward artificial flow from early headfold to six-somite stages. Artificial rightward flow reversed the pattern of *Pitx2* expression in LPM (left, red arrowhead) and that of ANE activity in crown cells, as detected by means of in situ hybridization of *LacZ* mRNA (middle) and X-gal staining (right). Scale bars, 100 μ m.

Fig. 3. Calcium signal is essential for L-R symmetry breaking in the node crown cells. (A) Activity of ANE is disrupted when Ca^{2+} signal blockers are treated. Two pathways that lead to Ca^{2+} release from endoplasmic reticulum were examined (B). L > R asymmetric ANE activity is maintained in control and Ruthenium Red-treated embryos, whereas it is disrupted with Gd^{3+} , 2-aminoethoxydiphenyl borate (2-APB), or Thapsigargin (TG). Scale bar, 50 μ m. (C) The effects of each reagent on L-R asymmetric ANE activity are summarized. The numbers of embryos showing each pattern are indicated.



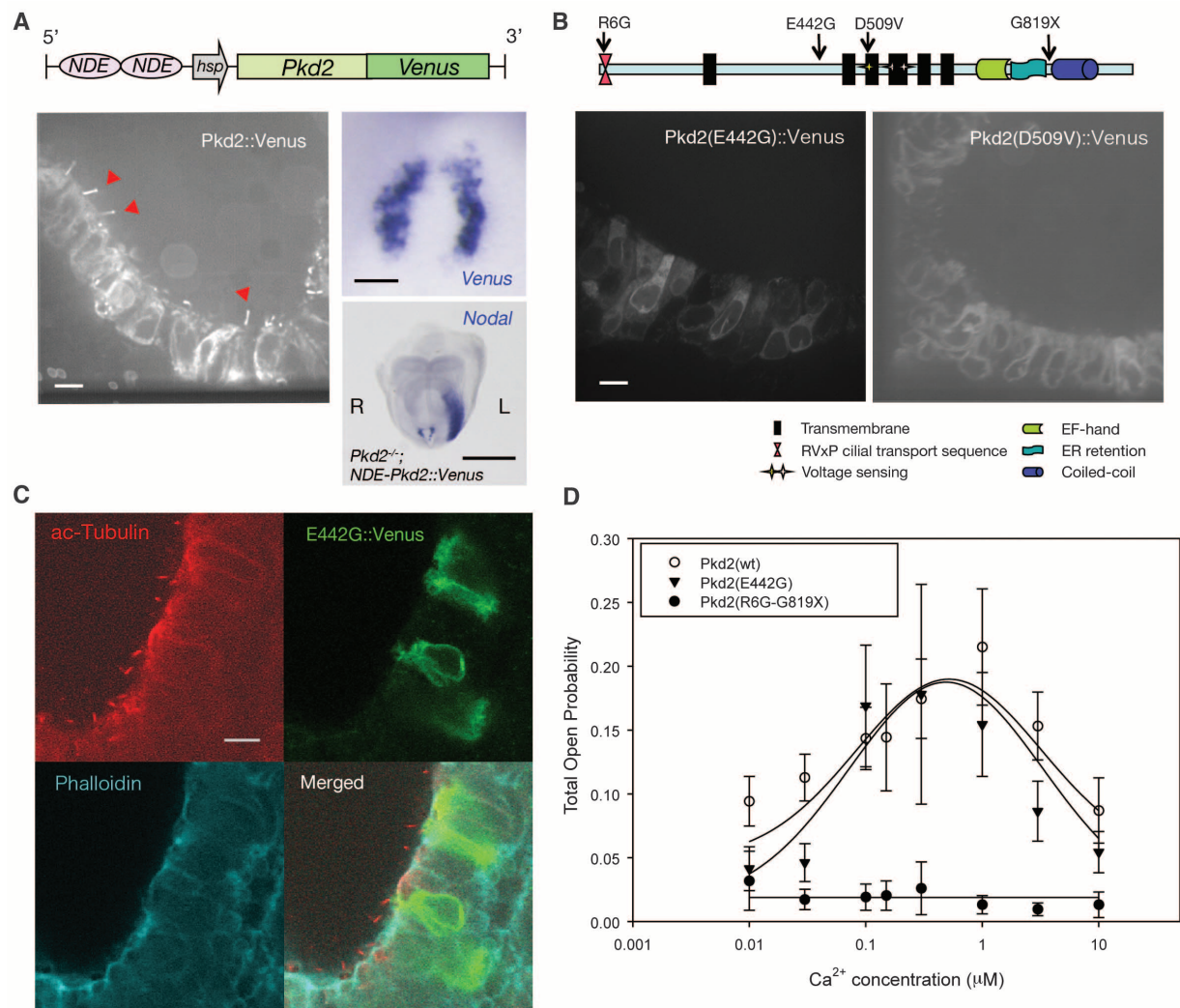


Fig. 4. Ciliary localization of Pkd2 is required for correct L-R determination. (A) Schematic representation of NDE-driven *Pkd2::Venus* transgene is shown on the top. Crown cell-specific expression of the transgene in an E8.0 embryo was confirmed by means of whole-mount in situ hybridization with a *Venus* probe (top right). Scale bar, 50 μm . Left-sided expression of *Nodal* was restored in *Pkd2*^{-/-};*NDE-Pkd2::Venus* embryo (bottom right), suggesting that *Pkd2::Venus* is functional. Scale bar, 500 μm . *Pkd2::Venus* protein is preferentially localized to cilia (left, red arrowheads). Scale bar, 10 μm . (B) Transgenic embryos expressing

Pkd2(E442G)::Venus or *Pkd2(D509V)::Venus* in crown cells. Both proteins are unable to localize to cilia. Scale bar, 10 μm . (C) Localization of *Pkd2(E442G)::Venus* was confirmed by means of immunofluorescence staining with antibodies to acetylated tubulin (red), to Venus;green fluorescent protein (GFP) (green), and to phalloidin (blue). Scale bar, 10 μm . (D) Open probability of *Pkd2*(wt), *Pkd2(E442G)*, and *Pkd2(R6G-G819X)* channels in the presence of increasing cytosolic Ca^{2+} concentration. *Pkd2(E442G)* retains normal channel activity, whereas *Pkd2(R6G-G819X)* loses it. Error bars represent $\pm\text{SEM}$.

in LPM was examined. Whereas *Kif3a*^{-/-} embryos (5 of 5) failed to respond to the rightward flow, the *Kif3a*^{-/-};*NDE-Kif3a* embryos (3 of 3) did respond by showing right-sided *Pitx2* expression in LPM (Fig. 5, B and C). These results thus demonstrated that nodal flow is indeed sensed by crown cell cilia located at the edge of the node. Our results indicate that the fluid flow in the node is sensed by cilia of perinodal crown cells via ciliary-localized Pkd2. Many proteins are known to be localized to primary cilia, and they are implicated in cilium-mediated signaling. However, the role of their ciliary localization has not been rigorously established. Identification of *Pkd2(E442G)*—a L-R defect-causing mutant form of *Pkd2* that retains Ca^{2+} channel activity yet is unable to localize to the cilia—

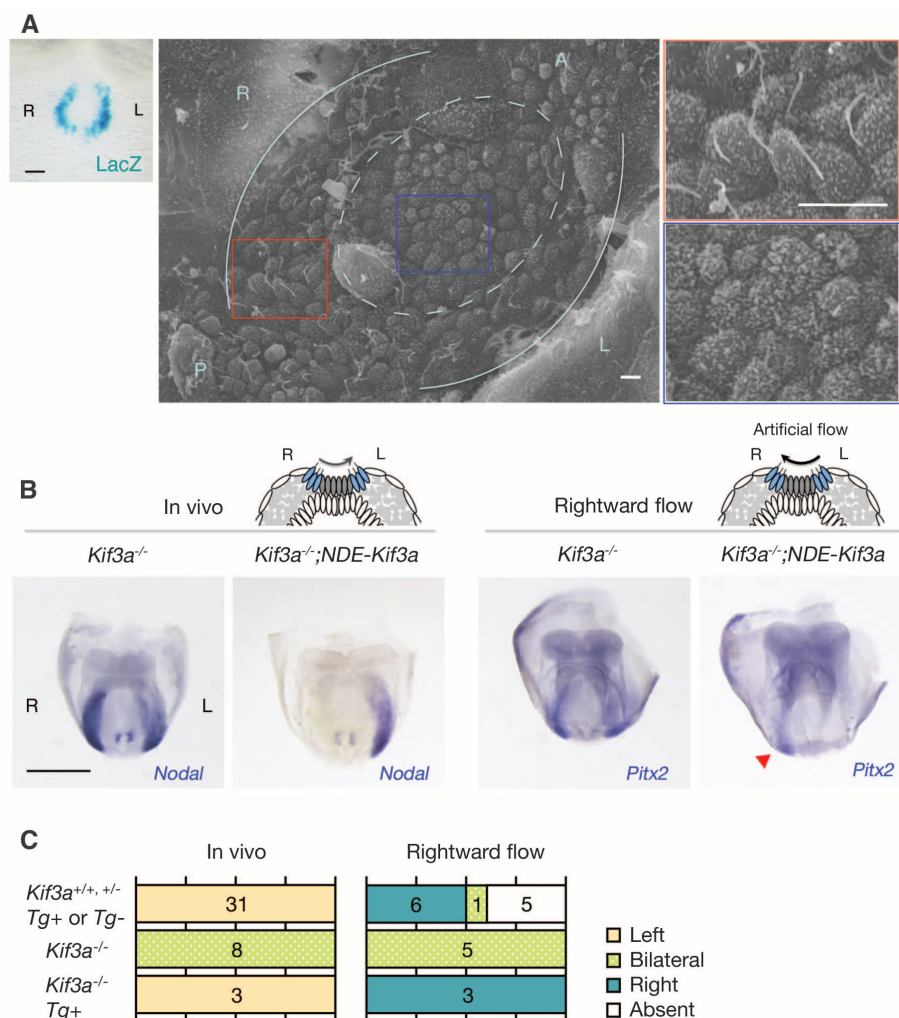
Table 1. Properties of various Pkd2 mutants. The relation between ciliary localization, Ca^{2+} channel activity, ability of L-R rescue, and interaction with Pkd11 is summarized for various Pkd2 mutants. ND, not determined.

Name	Mutation	Cilia localization		L-R rescue	Ca^{2+} channel property	Interaction with Pkd11
		Node	LLC-PK1			
R6G	R ₆ VxP point mutation	Yes	ND	Yes	ND	ND
$\Delta(5-73)$	R ₆ VxP deletion	Yes	ND	ND	ND	ND
R6G, G819X	Δ coiled-coil domain	No	ND	No	Impaired	ND
E442G	Irm4 (18)	No	No	No (18) (ND)	Normal	Yes
D509V	Human D511V (4)	No	No	No	Impaired (4)	ND

provides the direct evidence showing that ciliary localization of a protein is indeed essential for cilium-mediated signaling.

Several questions remain unanswered. It is still not clear what the cilia sense in the breaking of L-R symmetry: Do they sense flow-transported

Fig. 5. Nodal flow is sensed by the cilia of crown cells. **(A)** An E8.0 transgenic embryo harboring *NDE-Kif3a-IRES-LacZ* was stained with X-gal, revealing crown cell-specific expression of the transgene (left). Scale bar, 50 μ m. In SEM of the node of an E8.0 *Kif3a*^{-/-} embryo with *NDE-Kif3a*, cilia are apparent at the edge (red box) but not at the center (blue box) of the node. Pale blue lines indicate the border between the endoderm and crown cells, with the dotted circle enclosing pit cells. The boxed regions on the left are shown at a higher magnification on the right. Scale bars, 5 μ m. **(B)** Expression of L-R marker genes in embryos of the indicated genotypes that were examined at E8.0 (in vivo) or cultured under the influence of a rightward artificial flow before analysis. *Pitx2* expression pattern of the *Kif3a*^{-/-};*NDE-Kif3a* embryo responded to the flow and is right-sided (red arrowhead). Scale bar, 500 μ m. **(C)** The numbers of embryos showing each pattern of gene expression are summarized.



chemicals or flow-generated mechanical forces? Pkd2 is localized to the primary cilium of kidney cells and vascular endothelial cells and mediates mechanosensation (25, 26). Circumstantial evidence, including our recent observation that as few as two rotating cilia are sufficient for L-R symmetry breaking (24), favors the possibility that crown cell cilia sense mechanical force. Also unknown is how cilia signal to *Cer12*, the major target of the signal transmitted by nodal flow and Pkd2. Our results support the previous proposal (13, 27) that there are two populations of cilia in the node, motile and immotile cilia, and that the former generate nodal flow, whereas the latter sense the flow. Given that most cilia of crown cells are immotile at the time when L-R symmetry is broken, it is likely that the flow is sensed by immotile cilia, although we are not able to rigorously exclude the possibility that motile cilia of crown cells also sense the flow. Further studies with diverse approaches are needed to address these issues.

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Acknowledgments: We thank Y. Cai for YCB9 antibodies to Pkd2; S. Somlo for very helpful comments on Pkd2 function; D.P. Norris for Pkd111-CC-GFP plasmid; and A. Fukumoto, Y. Ikawa, K. Miyama, K. Mochida, H. Nishimura, and S. Ohishi for technical assistance. This work was supported by a grant from CREST of the Japan Science and Technology Corporation to H.H., an NIH grant P30 DK090744 to B.E.E., and Human Frontier Science Program grant ST00246/2003C and the Deutsche Forschungsgemeinschaft grant PE 853/2 to P.P. S.Y. was supported by a fellowship from the Japan Society for the Promotion of Science for Japanese Junior Scientists. I.Y.K. was supported by the American Heart Association Postdoctoral Fellowship R10682.

Supplementary Materials

www.sciencemag.org/cgi/content/full/science.1222538/DC1
Materials and Methods
Figs. S1 to S14
References
Movies S1 to S3

28 March 2012; accepted 20 August 2012
Published online 13 September 2012;
10.1126/science.1222538

Strain Tuning of Individual Atomic Tunneling Systems Detected by a Superconducting Qubit

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In structurally disordered solids, some atoms or small groups of atoms are able to quantum mechanically tunnel between two nearly equivalent sites. These atomic tunneling systems have been identified as the cause of various low-temperature anomalies of bulk glasses and as a source of decoherence of superconducting qubits where they are sparsely present in the disordered oxide barrier of Josephson junctions. We demonstrated experimentally that minute deformation of the oxide barrier changes the energies of the atomic tunneling systems, and we measured these changes by microwave spectroscopy of the superconducting qubit through coherent interactions between these two quantum systems. By measuring the dependence of the energy splitting of atomic tunneling states on external strain, we verify a central hypothesis of the two-level tunneling model for disordered solids.

At low temperatures (below 1 K), specific heat and thermal conductivity of amorphous solids deviate markedly from predictions based on long-wavelength phonons in a three-dimensional elastic solid (1). To explain this behavior, which is independent of chemical composition, it has been suggested by Phillips (2) and Anderson *et al.* (3) that in the irregular structure of a disordered solid, some atoms or small groups of atoms experience a potential landscape that allows them to occupy two almost-equivalent sites. This situation is modeled by two potential wells of similar depth separated by an energy barrier (Fig. 1). For plausible parameter values, the tunneling of the atomic entity gives rise to low-energy excitations that are effective in the temperature range below 1 K. Disregarding higher-level vibrational excitations, this is equivalent to a two-level tunneling system (TLS) with an energy splitting

$$E = \sqrt{\Delta_0^2 + \Delta^2}, \text{ where } \Delta_0 \text{ is the tunneling}$$

energy related to the overlap of the wave functions of the particle in either of the two wells, and Δ is the energy difference between the two minima of the double-well potential (Fig. 1). In an amorphous solid, the values of both Δ_0 and Δ have broad distributions. Phenomenologically, the low-temperature thermal anomalies mentioned above and further dynamical properties can be accounted for if an essentially constant density of TLS states $n(E)$ is assumed (4–6).

Atomic TLSs are believed to be a source of noise and decoherence for various solid-state circuits (7–10), as well as nanomechanical (11, 12) and optomechanical devices (13); ensembles of

TLSs are thought to be the major general source of decoherence for superconducting qubits and microwave resonators. In particular, the quantum coherence times of qubits based on Josephson junctions (JJs) were found to be largely affected by the presence of TLSs located predominantly in the tunnel barriers of the junctions (7, 8), which are typically made of a 2- to 3-nm-thick layer of disordered aluminum oxide. Spectroscopic measurements indicate individual coherent TLSs resonantly interacting with the qubits (7, 14–16). Some TLSs exhibited coherence times much longer than those of the superconducting qubits (16–18) and, hence, can themselves be used as effective quantum memories (19) or computational qubits for testing quantum information protocols (20, 21).

However, these experiments with TLSs are limited by the fact that TLS properties can not be predicted or controlled at will (7, 14–21). An individual JJ qubit has its own random distribution of TLS frequencies. Individual coherent TLSs remain rather stable at temperatures below 1 K, but thermal cycling to room temperature completely changes TLS frequencies and their coupling strength to the qubit. These unpredictable properties represent a major obstacle to potential usage of coherent TLSs in hybrid quantum circuits.

Here, we report an experiment in which we tune the energy of coherent TLSs coupled resonantly to a JJ qubit. When varying a static strain field in situ and performing microwave spectroscopy of the junction, we observe continuously changing energies of individual coherent TLSs.

Information on how TLSs couple to strain fields comes mainly from ultrasonic measurements on glasses (4–6). However, these measurements only reveal the mean response of a huge number of TLSs, averaging over their individual coupling strengths and essentially ignoring the tensorial character of the strain field. General arguments (4, 22) suggest that coupling to strain fields oc-

curs mainly through a change of the asymmetry Δ of the double-well potential (Fig. 1), whereas changes of Δ_0 are negligible. The Hamiltonian of a TLS in the localized representation may thus be written as

$$H = \frac{1}{2} \begin{pmatrix} \Delta & -\Delta_0 \\ -\Delta_0 & -\Delta \end{pmatrix} + \frac{1}{2} \begin{pmatrix} \delta\Delta & 0 \\ 0 & -\delta\Delta \end{pmatrix} \quad (1)$$

with $\delta\Delta = 2\gamma\epsilon$, where $\gamma = 1/2\partial\Delta/\partial\epsilon$ describes the variation of the asymmetry with the (dimensionless) strain field ϵ (23). For static strain, the two terms of the Hamiltonian may be added (that is, the strain-induced $\delta\Delta$ adds to the intrinsic asymmetry Δ) to give, in the representation of the then-valid eigenstates, an energy splitting (23)

$$E = \sqrt{\Delta_0^2 + (\Delta + \delta\Delta)^2} \quad (2)$$

To verify this $E(\epsilon)$ dependence, we tuned the TLS asymmetry energy $\Delta + \delta\Delta$ by bending the sample while tracking the TLS energy E with a resonantly coupled JJ qubit.

To detect individual TLSs, we used a Josephson tunnel junction operated as a phase qubit (24). The measurement circuit (Fig. 2) consists of a superconducting loop interrupted by a JJ. The phase qubit forms an LC -resonator, where L comprises the loop inductance and the nonlinear inductance of the JJ, and C is the junction capacitance. Quantum dynamics of the qubit is determined by the charge on the capacitor and the phase drop across the junction acting as conjugated variables. Because the charge is subject to large quantum uncertainties caused by the relatively large capacitance, the qubit states $|0\rangle$ and $|1\rangle$ are the two phase eigenstates of lowest energy in a metastable potential well. The energy difference of these two states can be tuned by applying an external flux bias Φ_{ext} to the qubit.

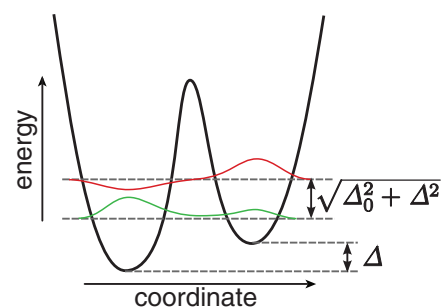


Fig. 1. Sketch of a double-well potential. The figure shows the two eigenstates with energy splitting $E = \sqrt{\Delta_0^2 + \Delta^2}$ and respective wave functions, built from the two localized states as symmetric and antisymmetric combinations. The tunneling energy Δ_0 depends on microscopic details and may be written as $\Delta_0 = \hbar\Omega\exp(-\lambda)$, where Ω is a typical vibrational frequency, $\lambda = d\sqrt{2mV_0}/\hbar$, m is the mass of the tunneling particle, d is the distance between the two potential wells along the appropriate configuration coordinate, $V_0 \gg \hbar\Omega$ is the potential barrier, and $\hbar$ is Planck's constant divided by 2π .

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In a qubit spectrum (Fig. 3A), for each value of Φ_{ext} , microwave pulses of varying frequency are applied to the qubit, which initially has been prepared in its ground state. The pulses are sufficiently long to generate an equal population probability of the two qubit states if the microwave frequency is at resonance, whereas off-resonance microwave pulses leave the qubit in its ground state. To read out the qubit, a bias flux pulse of 1-ns duration is applied to lower the potential barrier of the metastable well such that only the excited qubit state has a high probability of escaping from the well. This escape is associated with a change of the flux in the qubit loop, which is finally detected by an inductively coupled superconducting quantum interference device (SQUID) operated as a magnetometer. The spectrum thus reflects the probability $P(|1\rangle)$ of the qubit to be in state $|1\rangle$ after applying the microwave pulse.

The spectrum of the JJ qubit (Fig. 3A) clearly shows avoided level crossings, which have been attributed to the qubit's coherent interaction with individual TLSs (7, 14–16). The coupling strength v equals the frequency gap in the avoided level crossing (Fig. 3A, inset), and the center of the anticrossing corresponds to the TLS energy $E = hf_{\text{TLS}}$, where h is Planck's constant and f_{TLS} is the TLS resonance frequency. Although alternative theoretical models of two-level systems have been proposed to explain the avoided level crossings observed in qubit spectroscopy data (25–28), their microscopic origin can be explained most consistently by atomic two-level tunneling systems carrying an electric dipole moment (24, 29) and being located inside the aluminum oxide tunnel barrier of the junction (23). For our study, we chose a sample from the first generation of flux-biased phase qubits (7). These qubits were fabricated with a relatively large qubit junction of area of $32 \mu\text{m}^2$, which means that many TLSs are present to which the qubit may couple (23).

We quantitatively estimated the strain fields necessary to generate noticeable changes of the TLS energy. Figure 3A shows that the corresponding frequency changes (δf_{TLS}) should

be comparable or larger than the avoided level splitting; that is, on the order of 100 MHz. Going back to Eq. 2 and knowing from acoustic experiments on glasses that γ assumes values on the order of 1 eV, we find that strain fields (ϵ) on the order of 10^{-7} should be sufficient. On the surface of a 0.4-mm-thick substrate, such a deformation can be achieved by bending to a curvature with a radius of ~ 2 km; in other words, our 6.2-by-6.2- mm^2 substrate should bulge in the center by ~ 2.4 nm relative to the edges (23).

Our silicon substrate with the circuit that we studied is suspended in a notch with two opposite edges held down, whereas the center is supported and displaced by a stacked piezo element. For the force to act at a well-defined point, a small gold-plated zirconia sphere was glued on top of the piezo stack (fig. S1). Low-temperature calibration of the piezo actuator, together with numerical simulations of the chip's deformation, yields the translation factor between the strain ϵ in the vicinity of the qubit and the applied piezo voltage V_p to be $\epsilon/V_p \approx 7.8 \cdot 10^{-7}/\text{V}$ (23). All data reported in the remainder of this paper were measured at a base temperature of 45 mK.

Qubit spectra for different piezo biases (Fig. 3C) show shifts of the avoided level crossings, whereas the qubit's resonance frequency remains unaffected. Because TLSs are expected to have a broad range of coupling strengths and no preferred orientation with respect to the strain field, different avoided level crossings change their frequencies at different rates.

To investigate the changes of TLS frequencies with applied piezo voltage more systematically, we developed the following procedure. Instead of taking whole spectra as those shown in Fig. 3A, we measured the value $P(|1\rangle)$ at the expected qubit resonance frequency f_q , known to vary as $\sqrt{I_c^2 - I^2}$, for every given flux (here, I_c is the critical current of the JJ, and $I \propto \Phi_{\text{ext}}$ is the

current induced in the qubit loop). Once the qubit is resonant with a TLS, the emerging avoided level crossing decreases the value of $P(|1\rangle)$ measured at f_q (Fig. 3B). A comparison with Fig. 3A shows that minima in $P(|1\rangle)$ correspond to avoided level crossings.

Figure 4A shows a series of frequency scans taken similarly to those in Fig. 3B, as a function of the piezo voltage V_p . The lighter color marks frequencies where the qubit is at resonance with a TLS. Here, the strain-tuned energy splitting of any TLS can be tracked continuously. From additional measurements in a frequency range between 11 and 13.5 GHz, we identified 41 individual TLSs. The distribution of rates at which the TLS frequencies change with piezo voltage, $\delta f_{\text{TLS}}/\delta V_p$ (Fig. 4B, inset), closely resembles a Gaussian curve centered at zero. This is expected, because, due to the random location and orientation of TLSs in the disordered AlO_x tunnel barrier of the JJ, no particular sign of the TLS frequency change is preferred when mechanical strain is applied. In terms of deformation potential γ , this distribution extends to values of $\gamma \sim \pm 0.4$ eV.

The most notable feature in Fig. 4A is a broad and curved TLS trace, whose frequency changes nonmonotonically with applied mechanical strain. The corresponding TLS resonance frequency as a function of the piezo voltage is again plotted in Fig. 4B; the data are fit to the central hypothesis of the TLS model (Eq. 2), which predicts the TLS energy splitting E to depend hyperbolically on the asymmetry energy. In our case, the asymmetry is given by $\Delta + \delta\Delta$ and defined as $\Delta + \delta\Delta = \gamma V(V_p - V_0)$. The fit to Eq. 2 shown in Fig. 4B yields the deformation potential of this particular TLS to be $\gamma = h \cdot 4.72 \cdot 10^{13}$ Hz or 0.20 eV (23). We conclude that the applied strain moves this particular TLS through its symmetry point at which both localized atomic configurations have the same energy. Although this behavior is expected

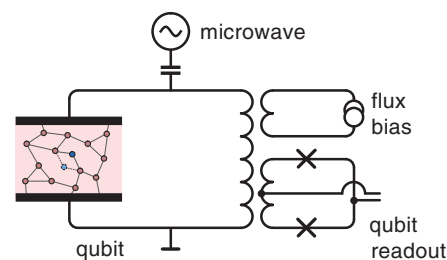


Fig. 2. Schematics of the sample circuit. The qubit consists of a superconducting loop interrupted by a JJ. The JJ is modeled as a Josephson element shunted by the intrinsic capacitor C . The sketch indicates the disordered structure of the JJ's tunnel barrier with an embedded TLS. The qubit is controlled by a microwave source and by the external flux, and it is read out by a dc-SQUID.

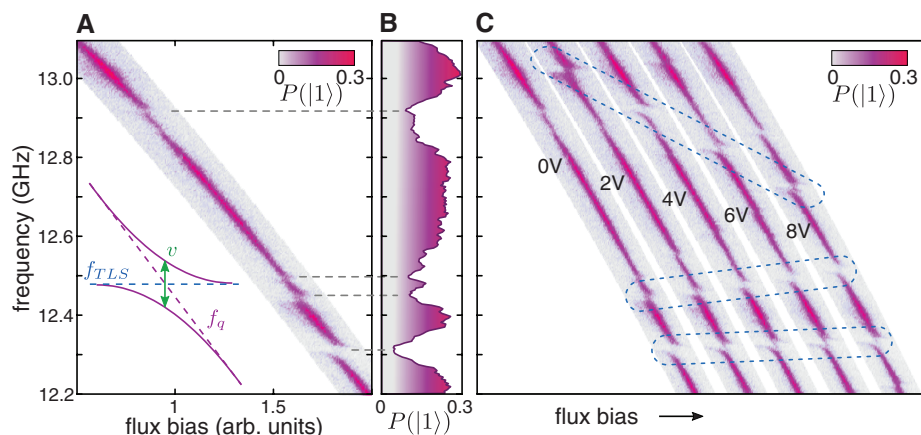


Fig. 3. Qubit spectroscopy. (A) Qubit spectrum as a function of the external flux Φ_{ext} . The avoided level crossings (shown schematically in the lower inset) correspond to individual TLSs coupled to the qubit. The size of the splitting v equals the coupling strength. (B) Probability of measuring the excited qubit state at its expected resonance frequency f_q , known to vary as $\sqrt{I_c^2 - I^2}$, for every given flux (here, I_c is the critical current of the JJ, and $I \propto \Phi_{\text{ext}}$ is the current induced in the qubit loop). Once the qubit is resonant with a TLS, the emerging avoided level crossing decreases the value of $P(|1\rangle)$ measured at f_q (Fig. 3B). A comparison with Fig. 3A shows that minima in $P(|1\rangle)$ correspond to avoided level crossings. (C) Qubit spectra similar to (A), taken at five different values of the piezo voltage V_p . The spectra are shifted along the flux axis for clarity.

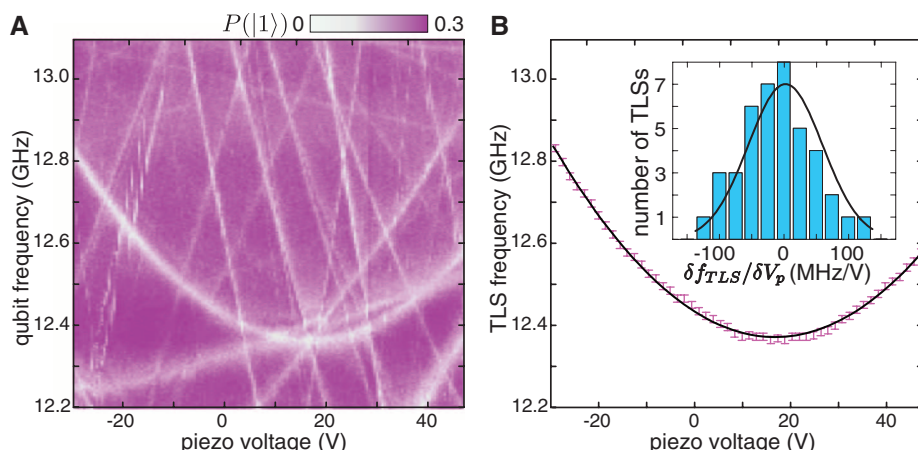


Fig. 4. Tuning two-level systems by strain. **(A)** At each value of V_p , a spectroscopic line like that shown in Fig. 3B is recorded. The resonance frequencies of the TLSs ($f_{\text{TLS}} = E/h$ as a function of V_p) appear lighter in color. The frequency dependence of the TLS showing a broad nonmonotonic behavior is extracted and fitted to a hyperbola given by Eq. 2 in **(B)**. The error bars correspond to the width of the resonance dips. The inset in **(B)** shows the distribution of the linear frequency changes of the TLSs with respect to the voltage change; the solid line shows a Gaussian distribution for comparison.

for all TLSs, it can only be observed for TLSs that have their symmetry-point energy splitting in the accessible range limited by the tunability of the phase qubit. The other TLSs have tunnel energies Δ_0 much smaller than what is experimentally accessible; hence, only the nearly linear tails of their energy hyperbolas are observed. Data taken in the same way on another sample (fig. S4) (23) reveal five TLS traces obeying a hyperbolic frequency dependence on strain, showing that this is a common property of TLSs.

Another interesting trace in Fig. 4A, appearing between -30 and -10 V, indicates a TLS whose resonance frequency shifts at the rate of ~ 50 MHz/V and jumps randomly, within hours, between two traces ~ 100 MHz apart. Most likely, the local potential landscape of the coherent TLS under observation changes abruptly due to a nearby incoherent TLS fluctuating slowly and randomly between its localized states.

The basic idea of the above experiment has some similarity to earlier experiments (30, 31) in which static strain was used to change the dwell times of individual incoherent TLSs (two-level fluctuators) in disordered metallic nanostructures. The experiments here are performed with individual coherent TLSs that have tunneling energies Δ_0 on the order of $k_B T$ (where k_B is the Boltzmann constant and T is temperature) and asymmetry energies Δ tunable by strain from values on the order of $k_B T$ to zero. The TLS energies E are measured directly, and their strain dependence given by Eq. 2 is confirmed.

All results presented above are explained readily by the tunneling model and, therefore, provide firm evidence of the hypothesis that atomic TLSs are the cause of avoided level crossings in the spectra of JJ qubits. Mechanical strain offers a handle to control the properties of coherent TLSs, which is crucial for gaining knowledge about their physical nature. Our method can also be used to

tune TLS frequencies away from the qubit resonance and, thus, to optimize the coherence of a qubit circuit at its operation point. Moreover, the knowledge of how individual coherent TLSs are modified by strain opens an opportunity to manipulate TLSs via ultrasonic excitation.

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Acknowledgments: We thank M. Ansmann and J. M. Martinis (University of California, Santa Barbara) for providing the sample we measured in this work and J. H. Cole, E. Demler, Y. M. Galperin, M. D. Lukin, J. M. Martinis, R. McDermott, J. E. Mooij, C. Müller, A. Shnirman, and J. Wrachtrup for fruitful discussions. This work was supported by the DFG and the State of Baden-Württemberg through the DFG CFN, as well as by the European Union project SOLID.

Supplementary Materials

www.sciencemag.org/cgi/content/full/338/6104/232/DC1
Supplementary Text
Figs. S1 to S4
References (32–35)

25 June 2012; accepted 6 September 2012
10.1126/science.1226487

Observation of Resonances in Penning Ionization Reactions at Sub-Kelvin Temperatures in Merged Beams

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Experiments have lagged theory in exploring chemical interactions at temperatures so low that translational degrees of freedom can no longer be treated classically. The difficulty has been to realize in the laboratory low-enough collisional velocities between neutral reactants to access this regime. We report here the realization of merged neutral supersonic beams and the manifestation of clear nonclassical effects in the resulting reactions. We observed orbiting resonances in the Penning ionization reaction of argon and molecular hydrogen with metastable helium, leading to a sharp absolute ionization rate increase in the energy range corresponding to a few degrees kelvin down to 10 millikelvin. Our method should be widely applicable to many canonical chemical reactions.

Theoretical quantum chemistry suggests that at low-enough collision energies, where reactants' de Broglie wavelength approaches

the characteristic interaction length scale, quantum effects, such as tunneling through reaction or centrifugal barriers, will dominate the reaction

dynamics (1). So far, studies in this regime have largely been confined to theory because of the experimental challenge of achieving millikelvin collision energies. Among the reported observations of reactions at temperatures of 1 K or below (2–5), Ospelkaus *et al.* (4) presented evidence of nonclassical effects in the reaction of ultracold KRb molecules. In a different approach, Schreiner *et al.* (6) demonstrated that tunneling dominated isomerization reaction rates for molecules trapped in an argon matrix at 11 K. One of the most notable manifestations of quantum tunneling is the formation of scattering resonance states that can dramatically affect the reaction rate. Quasi-bound resonance states arise in the continuum portion of the spectrum, making them accessible to scattering experiments wherein the collision energy can be tuned. Detection of scattering resonances in reactions allows an accurate determination of the interaction potentials governing the collision dynamics. Such measurements constrain various theoretical models and methods, such as electronic structure calculations (that because of complexity always involve approximations) and molecular dynamics simulations that necessarily require quantum treatment at these low temperatures. Although dynamical Feshbach resonances have been measured in the differential cross-section of the $F + H_2$ reaction (7), so far tunneling resonances have not been observed in low-temperature reactive scattering.

We report here the observation of scattering resonances in the Penning ionization (PI) of argon and molecular hydrogen upon collisions with metastable helium atoms. PI is a fundamental reaction in nature between two neutral species leading to ionization by impact with an internally excited atom or molecule (8). This process is central to plasma chemistry (9) and has many applications, including surface characterization studies (10) and mass spectroscopy (11). PI falls

into a large class of processes in physics that involve a discrete electronic state embedded into a continuum of states to which it is coupled, resulting in an autoionization process (12). However, the autoionizing collisional complex is only formed during a collision, and nuclear dynamics play a major role in PI reactions. We show that quantum effects in the nuclear motion strongly affect the ionization rate by observing sharp peaks arising from the formation of metastable collision complexes via quantum tunneling through an angular momentum barrier. Such a behavior is governed by the long-range interactions between the colliding species, is general to neutral-neutral collisions in the low-energy range, and has been predicted to occur in many reactive systems, including chemical (13–17) as well as PI reactions (18). The experimental breakthrough that enabled our observations was a merged beam approach that tuned collision temperatures down to 10 mK, despite the use of conventional supersonic molecular and atomic beams without any additional cooling stage. At 10-mK collision temperature, the corresponding de Broglie wavelength in the case of molecular hydrogen PI is 27 nm. The temperatures that we achieve are more than three orders of magnitude lower than those attainable in state-of-the-art systems featuring collision energy tunability. The advantage of reducing relative velocity, or collision energy, by merging two beams was recently theoretically investigated by Wei *et al.* (19), and we realized this idea by bending the trajectories in one of the beams with use of a curved magnetic quadrupole guide.

The history of angular momentum barrier tunneling, also called orbiting resonances, dates far back to the origins of collision theory. Boltzmann postulated that a collisional metastable complex forms during recombination reactions via an interaction with a third body (20). This possibility was later ruled out as improbable. Bunker recognized that the metastable intermediate state, formed by the colliding atoms and molecules, can be trapped by the potential barrier, which emerges from the contribution of the centrifugal potential (21). In the case of elastic scattering, the

effect of the orbiting resonances on the elastic collision cross-section has been experimentally measured by making a very clever choice of collision partners, leading to a system with a low reduced mass and a weak van der Waals interaction strength (22–24). At these favorable conditions, orbiting resonances are formed at high collision energies equivalent to about 10 K. Shape resonances have been detected in the elastic scattering of laser-cooled atoms as well (25, 26). Recently, Chefdeville *et al.* (27) observed resonance structure in inelastic scattering of CO molecules with molecular hydrogen above the excitation threshold temperature of 5 K.

Although the appearance of resonances in low-energy collisions is a rule rather than an exception, observations are confounded by the lowest collision energies available in experiments, as well as the requirement for a resolution high enough to discern individual resonance states. Practically, it is necessary to scan rates in an energy range corresponding to temperatures from a few degrees kelvin to a few millikelvin. Below several mK, only the *s*-wave scattering channel remains open, and the Wigner threshold law (28) reestablishes monotonic rate behavior.

Among the many available methods that permit tuning of the collision energy, none have reached temperatures sufficiently low to enable routine observation of resonances in the cold regime. Two ways to induce cold collisions are to perform the experiment in a cryogenic cell (29, 30) or within a uniform supersonic expansion with carefully controlled temperature and density (31, 32). Another school of thought, motivated by the pioneering experiments conducted by D. Herschbach and Y. T. Lee (33) involving the use of two crossed supersonic beams, aims to control the collision energy by varying the angle of collision or the velocity of one of the beams. Recently, adjustment of the crossing angle has been used to measure a reaction cross-section down to a temperature of 5 K (34). Meijer and colleagues used pulsed electric field decelerated supersonic beams to study inelastic scattering (35). Inelastic scattering experiments were also performed with magnetically trapped atoms and molecules (36, 37) with the lowest temperature reported at 5 K (38).

In our approach, we were able to break through the 1-K collision temperature threshold by merging two fast supersonic beams: Relative velocity vanishes in a moving frame of reference when beams of equal velocities merge. Similar methods have been used in ion chemistry for almost two decades (39). In contrast to charged particles where trajectories can be easily controlled via interaction with modest electric fields, the neutral particles' center of mass motion is far more challenging to control. We used the Zeeman effect in order to direct paramagnetic species through a curved magnetic quadrupole guide. At the exit of the quadrupole, the paramagnetic particle beam merges with another supersonic beam that has travelled in a straight line from a separate supersonic source. There is no restriction on the nature

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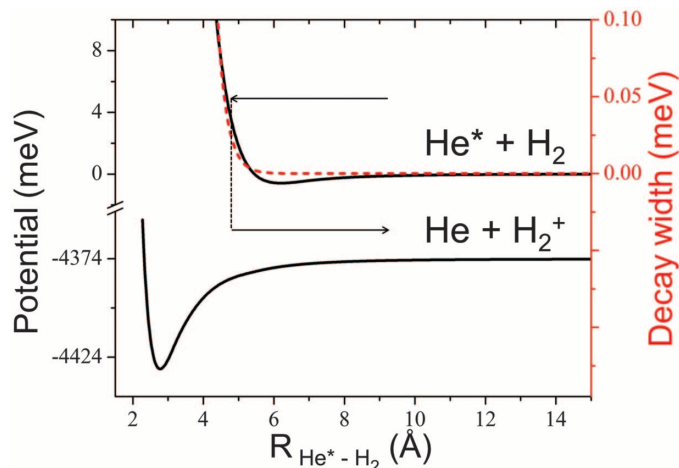
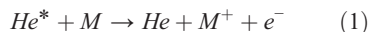


Fig. 1. The spherical portion of the interaction potentials involved in the PI reaction of metastable He (3S) and molecular hydrogen. The entrance channel includes both the real part of the potential (top solid black line) as well as autoionization width (dashed red line). The bottom black curve represents the ion-neutral exit-channel interaction potential.

of the second beam, and any atom or molecule that can be entrained into the supersonic beam could be used in principle. By varying the relative velocities between the two beams, we continuously tune collisional energies from 350 K down to 10 mK. The lower bound for collision energy is unexpected. One would naïvely expect the lowest collision temperature we can achieve to be limited by the temperature of the hottest beam among the two, in the range of 0.1 to 1 K. The reduction in energy occurs because of longitudinal momentum compression in phase space during free propagation. Our nozzle-opening duration is much shorter compared with the propagation to the detector time, and the initial spherical phase space distribution deforms assuming a cigar shape.

In our study of low-energy PI reactions, we merged a supersonic beam of metastable helium with a supersonic beam containing either argon or molecular hydrogen. The excited 3S state of helium has an energy of 19.8 eV above the

ground state. When a metastable helium atom collides with another atom or molecule with an ionization energy lower than 19.8 eV, a charge-transfer process takes place whereby an electron from the neutral species jumps into the vacancy of the 1s orbital of helium, coinciding with the ejection of an electron from the 2s orbital to the continuum (Eq. 1)



PI has been studied in detail at higher energies, with many interesting results summarized in a review article by Siska (40). PI reactions have different entrance and exit channels (Fig. 1). In the entrance channel, the interaction between metastable helium and another atom or molecule can be described by using a complex optical potential. The real part of the interatomic potential contains the appropriate long-range van der Waals interaction, the leading term of which scales as R^{-6} , where R is the distance between the metastable

He atom and the colliding atom or molecule. At short distances the repulsion term takes over, whereas at intermediate distances there is a shallow van der Waals well. The complex part of the potential $\Gamma(R)/2$ is related to the ionization probability at a given internuclear distance. Because the charge transfer probability decays rapidly with separation, it is usually modeled by a single exponential term. Electronic motion is much faster compared with nuclear motion, and the autoionization process can be viewed as a vertical process within the Born Oppenheimer approximation. The resulting ion and neutral helium interaction is described by a neutral-ion potential surface in the exit channel.

A schematic of the experimental system is shown in Fig. 2A. The pulsed metastable helium supersonic beam was created by using an Even-Lavie valve (41) cooled down to 55 K. Immediately after the valve there is a dielectric barrier discharge (42), which was used to excite the ground-state helium to the 2^3S level. The beam had a mean velocity of ~ 770 m/s with a standard deviation of 15 m/s, corresponding to a temperature of 50 mK in the moving frame of reference. The beam then passed through a 4-mm-diameter skimmer located 10 cm from our valve orifice and entered a 20-cm-long magnetic quadrupole, which had a 10° curve with a curvature radius of 114.7 cm. We created a quadrupole magnetic field by passing a current pulse through 1-mm diameter copper wires arranged in quadratures, as shown in Fig. 2B; each quadrature consisted of nine wires in a three-by-three pattern. At the peak current of 1000 A, the transverse quadrupole trap depth was about 2.7 K and 3 mm by 3 mm in size. Only low-field-seeking Zeeman sublevels were confined in two dimensions during the transit through the quadrupole guide. As such, the metastable helium beam leaving the magnetic quadrupole was 100% spin-polarized in a single quantum state with the projection of the total

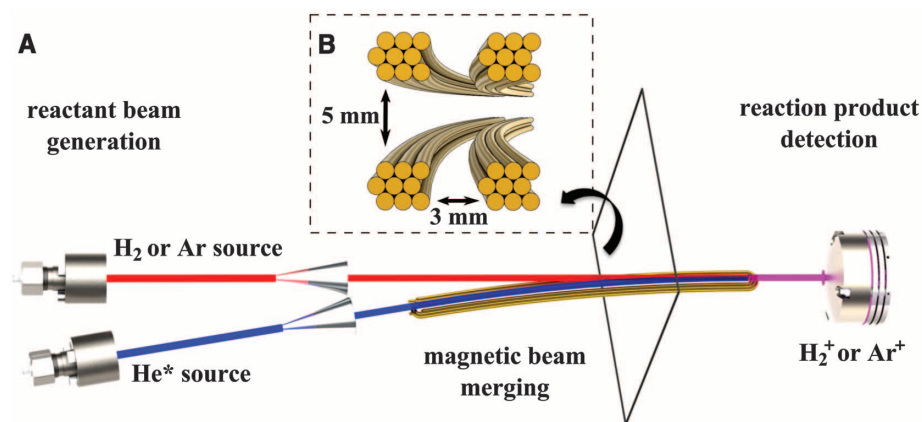
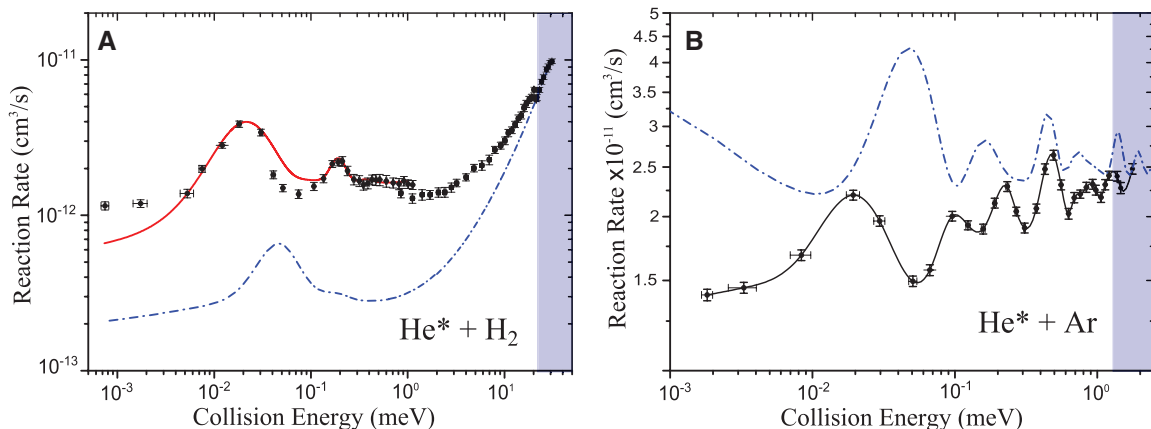


Fig. 2. (A) Schematic of the experimental system within the vacuum chambers, showing the two source supersonic valves followed by two skimmers, the curved magnetic quadrupole guide with its assembly, and the QMS entrance at the end. The blue beam is magnetically guided, whereas the red beam is unaffected. The merged volume is in purple. (B) A front view of the quadrupole guide.

Fig. 3. (A) Reaction rate measurements for (3S) He^* and H_2 PI are shown in black with error bars. The lowest collision energy achieved is 0.75 with standard deviation of 0.07 μeV , corresponding to 8.7 ± 0.8 mK temperature. Blue dash-dot line is the reaction rate calculated by using the most recent potential from (44). Red solid line is the calculated reaction rate by using the Tang-Toennies potential with parameters that give the best fit to our measured results. A second slower decay term is included in the imaginary part of the potential in order to adjust the reaction rate value at low collision energies. (B) Reaction rate measurements for (3S) He^* and Ar PI are shown in black with error bars, and the solid black line is a guide to the eye. The blue dash-dot line is the reaction rate calculated by using the most



recent experimental potential from (43). Shaded areas in both figures correspond to the energy range where our results overlap with the earlier measurements. The data points in (A) and (B) represent the mean $\pm$ standard deviations, where the reaction rate was determined by 50 measurements and the collision energy by 40 measurements.

angular momentum on the quantization axis, $m_J = 1$, originating from the 3S electronic state manifold. At the end of the quadrupole, the metastable beam merged with a second supersonic beam, also created with a temperature-controlled Even-Lavie valve, and collimated with a 4-mm skimmer. This second beam contained molecular hydrogen in one experiment and atomic argon in another, either as neat gases or seeded in a noble carrier gas. By changing the composition of the gas mixtures as well as the temperature of the valve (between 355 and 120 K), we changed the relative mean velocity between the beams from over 1000 m/s down to zero. At zero relative velocity between the beams, the residual collision energy stems from the finite velocity distribution of the supersonic beams within the collision volume.

We measured the time-of-flight signal for both reactant beams as well as the product. The metastable beam was measured by using a micro-channel plate, whereas the ground state beam was measured by using an ionizing quadrupole mass spectrometer (QMS). To measure the product ion, we turned off the ionizing element of the QMS in order to observe the ions formed in the chemi-ionization collisions. We first divided the ion signal by the product of the area of both neutral beams and then normalized by using the data from earlier high-collision-energy experiments (43, 44). Thus, we are able to present our results for hydrogen (Fig. 3A) and for argon (Fig. 3B) on the absolute reaction rate scale.

At higher collision energies, above 1 meV (11.5 K) and 20 meV (230 K) for the Ar and H_2 systems, respectively, our results are in very good agreement with earlier experimental measurements. A classical treatment of the collisional process is sufficient to explain the main results above this energy. The reaction rate falls at lower velocities because the inner classical turning-point position scales with energy. For lower energies, the clas-

sical turning point is positioned at larger internuclear separation where the ionization rate is lower. At about 5 and 1 meV in the cases of Ar and H_2 ionization, respectively, the reaction rate levels off because the decrease of the ionization rate is compensated by longer collision times.

At energies below 1 meV, collisions enter the quantum regime, and we observed a series of peaks in the total reaction rate as a function of energy. Peaks appeared in the cases of PI of both argon and molecular hydrogen. The number of peaks and the peak spacing scales with the reduced mass, indicating that the quantization of the internuclear coordinate is involved. To elucidate the origins of the resonant structures, we have performed numerical simulations of the collision process. We numerically integrated the Schrödinger equation with a complex potential and for each partial wave determined the complex phase shift from the asymptotic behavior of the numerical solution. In case of H_2 PI, we used the spherical symmetric part of the potential similar to the treatment presented by Toennies (24). The total ionization reaction rate is given by a weighted sum over the contribution of individual partial waves.

We used the most recent potential energy surfaces (44) constructed by using both ab initio calculation results and experimental data obtained at higher energies. The potential correctly captures the higher energy behavior yet fails to reproduce the positions of the resonance states (Fig. 3A). Because the interaction potentials of metastable helium with molecular hydrogen have been thoroughly investigated by using ab initio methods, we chose this system to try to fit the potential surface parameters so as to match the observed resonance positions. We used a Tang-Toennies potential (45), starting with parameters derived from the most recent potential surface. We were able to match the resonance positions only after we increased the van der Waals potential well depth by a factor of 1.8 and moved the well minimum position by 1 Å. Such sensitivity to the parameters of the potential surface illustrates the importance of measuring the resonance states in order to obtain the correct long-range interaction behavior. Furthermore, these experiments should stimulate theoretical investigations of the metastable helium-molecular hydrogen potential resulting from the system's simplicity, because it involves only four electrons and thus can serve as a benchmark for different ab initio methods. In addition to the mismatch in the positions of the resonance peaks, there was also a discrepancy, at low energies, of almost an order of magnitude between the measured and calculated reaction rates. The observed higher reaction rate can be reproduced in our calculations by the addition of a slower decay term to the imaginary part of the potential. This raises the question of whether the estimation of an autoionization width as a single decaying exponent is sufficiently accurate. This discrepancy could possibly arise from the underestimation of atomic and molecular orbital overlap at large separations.

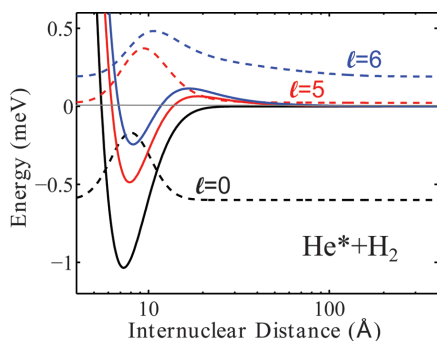


Fig. 4. Potentials and wave functions for the entrance channel of (3S) He^* and H_2 reaction. Potentials for different contributing angular momentum terms are shown as solid lines with the corresponding absolute values of wave functions as dashed lines. The black bottommost lines are for the potential with $l = 0$. Lines above that in red are for $l = 5$, and the topmost blue lines are for the $l = 6$ partial wave.

We have also calculated the resonance wave functions by using discrete variable representation (46) and complex scaling methods (47). In the case of molecular hydrogen PI, the resonant states appear in the partial waves associated with the total angular momentum $l = 5$ and 6 (Fig. 4). The orbiting resonance wave functions are highly delocalized, extending to more than 10 Å. The orbiting resonance associated with the $l = 6$ partial wave channel has an energy above the centrifugal barrier. Such a resonance is formed by a quantum reflection off, and not tunneling through, the angular momentum barrier.

The resonance wave functions illustrate the mechanism of low-energy resonant chemi-ionization. Classically, one would expect that collisions of particles at energies below the centrifugal barrier should not contribute to the ionization process. However, at collision energies that match positions of the resonance states, particles tunnel through the centrifugal barrier and become trapped. In such a case, the probability of finding colliding particles, described by the absolute value of corresponding wave function, is enhanced inside of the centrifugal barrier within the region where the autoionization rate is higher.

Our results manifest a quantum effect in ionization reactions below 1 K. We have observed orbiting resonances in the PI reaction of molecular hydrogen and argon with metastable helium that proceed by tunneling through an angular momentum potential barrier. As a result, we observed sharp peaks in the reaction rate measurements as a function of collision energy. The application of our method toward studying chemical reactions is straightforward. Atomic and molecular radical collisional partners are paramagnetic, and as such their beams can be manipulated by using our curved magnetic quadrupole. For example, a beam of atomic fluorine can be turned and merged with molecular hydrogen in order to study the canonical $F + H_2$ reaction in the cold regime where the reaction proceeds via tunneling. The theory of this chemical reaction suggests that resonances exist in the sub-kelvin energy range (48) and should be accessible by using our method.

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Acknowledgments: We acknowledge U. Even for many helpful discussions and sound advice. We thank R. Ozeri for most fruitful discussions; M. Raizen, R. Naaman, Y. Prior, and B. Dayan for a careful reading of the manuscript; and S. Assayag and M. Vinetsky from the Weizmann machine shop for assistance in designing and manufacturing of our vacuum chamber. This research was made possible, in part, by the historic generosity of the Harold Perlman Family. E.N. acknowledges support from the Israel Science Foundation and Minerva Foundation.

2 July 2012; accepted 6 September 2012
10.1126/science.1229141

An Ancient Core Dynamo in Asteroid Vesta

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The asteroid Vesta is the smallest known planetary body that has experienced large-scale igneous differentiation. However, it has been previously uncertain whether Vesta and similarly sized planetesimals formed advecting metallic cores and dynamo magnetic fields. Here we show that remanent magnetization in the eucrite meteorite Allan Hills A81001 formed during cooling on Vesta 3.69 billion years ago in a surface magnetic field of at least 2 microteslas. This field most likely originated from crustal remanence produced by an earlier dynamo, suggesting that Vesta formed an advecting liquid metallic core. Furthermore, the inferred present-day crustal fields can account for the lack of solar wind ion-generated space weathering effects on Vesta.

The terrestrial planets are thought to have formed from the successive growth and accretion of protoplanetary objects <1000 km in diameter (*1*). A fraction of these protoplanets have survived to the present day and include 4 Vesta, the second most massive asteroid (525 km mean diameter). In particular, Vesta's high density, primordial basaltic crust, and large size suggest that it is an intact remnant of the early solar system that escaped catastrophic collisional dis-

ruption (*2*). Vesta therefore provides an opportunity to characterize the building blocks of the terrestrial planets and to study the processes of planetesimal accretion and differentiation.

Meteorites of the howardite-eucrite-diogenite (HED) clan probably sample the crust and upper mantle of Vesta (*3*). Geochemical studies of HED meteorites suggest that Vesta has a fully differentiated structure, with a metallic core ranging from 5 to 25% of the total planetary mass (*4*) that formed within ~1 to 4 million years (My) of the beginning of the solar system (*5, 6*). Recent volume and mass constraints from the NASA Dawn mission provide evidence of a metallic core between 107 and 113 km in radius (*2*).

Vigorous advection in a molten metallic core may generate a dynamo magnetic field. Paleomagnetic studies of meteorites suggest that past dynamos may have existed on other asteroidal objects such as the angrite and the CV carbonaceous chondrite parent bodies (*7, 8*). These data

offer the possibility of studying the physics of dynamo action in a small-body regime not represented by active dynamos in the solar system today, in which Mercury is the smallest body with a known active dynamo (*9*). However, there has been no meteorite group for which evidence of dynamo action has been confidently established and that has been directly associated with a known, intact, asteroidal parent body.

Previous paleomagnetic studies have shown that many HED meteorites are low-fidelity recorders of magnetic fields because of their large (i.e., multidomain), low-coercivity magnetic minerals (*10*). Furthermore, these paleomagnetic studies generally lacked radiometric ages and thermochronometry. As a result, they came to no firm conclusions about the origin of magnetization identified in HED meteorites (*11*). Although dynamo-generated and even nebular fields were considered, other potential sources such as recent magnetic contamination and impact-generated fields could not be ruled out (*10, 12–14*). Here, we present a paleomagnetic study of ALHA81001 (ALH, Allan Hills), a meteorite found in Antarctica in 1981 with exceptional magnetic recording properties (*15*). We also present thermochronologic and petrographic data that constrain the origin of the meteorite's natural remanent magnetization (NRM).

The main-HED-group oxygen isotopic composition of ALHA81001 suggests that it originated on Vesta (*16*). As a eucrite, ALHA81001 has a basaltic composition and probably is a sample of the asteroid's upper crust. Our petrographic observations show that ~99 volume % of ALHA81001 has a fine-grained texture. A previous paleomagnetic study found that ALHA81001 has one of the most stable NRM records observed for any main-oxygen-isotope group HED

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(10). This is probably due to its very fine grain size, which has led to the formation of unusually high-coercivity ferromagnetic crystals [kamacite and iron sulfide (17)]. In particular, the width of plagioclase phenocrysts in the groundmass ALHA81001 indicates that the primary crystallization of the meteorite above 1150°C took place over the course of ~1 hour (18). However, the presence of ~0.4- μm -wide augite exsolution lamellae in host pigeonite grains suggests that the meteorite was reheated to between 800° and 1100°C, possibly due to burial in a hot ejecta blanket, and then cooled slowly over several hundred years (19). Because these temperatures are above the Curie point of FeNi minerals, any remanent magnetization in ALHA81001 must have been acquired during or after this slow-cooling episode. Furthermore, we observed no undulatory extinction in plagioclase phenocrysts in the groundmass (20), indicating that the meteorite escaped impact shock pressures above 5 GPa after this slow cooling (21).

We extracted 13 subsamples (a set of 9 and a second set of 4) from two parent samples of ALHA81001. The subsamples within the first and second sets were mutually oriented to within 5°

and 10°, respectively, whereas the two parent pieces were not mutually oriented. Subsamples taken from near the fusion crust produced by atmospheric passage have systematically different NRM directions from those of seven interior subsamples, whereas two subsamples extracted from between the fusion crust and the interior subsamples have intermediate NRM directions (Fig. 1). These data are consistent with heating and remagnetization of the meteorite's <2-mm-deep exterior during atmospheric passage and suggest that the interior was not strongly contaminated by hand magnets, weathering, or viscous remagnetization since the samples' arrival on Earth.

All 13 subsamples were progressively alternating field (AF) demagnetized or thermally demagnetized to characterize their NRM components. We observed three distinct components of magnetization in each subsample (Figs. 1, 2), with the exception of one fusion-crust subsample, which has only two components. A low-coercivity (LC) component is blocked up to a coercivity of 3 mT. Its unidirectionality across all subsamples and low coercivity are consistent with a viscous remanent magnetization (VRM) acquired since the meteorite's recovery from Antarctica in 1981. A medium-

coercivity (MC) and medium blocking temperature (MT) component, blocked from 3 to between 21 and 57 mT during AF demagnetization and up to 150°C during thermal demagnetization, is unidirectional across all subsamples (except one fusion-crust subsample that does not have an MC component). Its low blocking temperature and unidirectionality across all subsamples strongly suggest that it is a VRM acquired during the meteorite's residence in Antarctica. The intensities of both the LC and MC/MT components are also consistent with a VRM origin according to our laboratory VRM-acquisition experiments (22).

Fusion-crust and interior subsamples each carry distinct high-coercivity components (which we designate HCf and HC, respectively) that are blocked between 21 to 57 mT and 62 to >290 mT. The HC component is unidirectional throughout the interior subsamples (which are separated by up to 0.9 cm), and the HCf component is unidirectional across the fusion-crust subsamples, but the HC and HCf directions are mutually divergent (Fig. 1). Thermal demagnetization identified a high-temperature (HT) magnetization in the same direction as the HC component and blocked between 150° and >275°C, above which irreversible alteration of the magnetization carriers occurs (23).

Upon AF demagnetization, the HC magnetization decays linearly to the origin with remarkable stability as compared to the NRM observed in all previously measured HED meteorites (10, 12) (Fig. 2). Its AF demagnetization spectrum is most similar to that of an anhysteretic remanent magnetization (ARM) and differs from that of a strong field (280 mT) isothermal remanent magnetization (IRM) or pressure remanent magnetization (PRM) acquired in a laboratory field of 750 μT at a pressure of up to 1.8 GPa [an analog for shock remanent magnetization (SRM) (22)]. All of these characteristics suggest that the HC/HT component is a thermoremanent magnetization (TRM) acquired during cooling in a magnetic field (24).

The $^{40}\text{Ar}/^{39}\text{Ar}$ plateau age of ALHA81001 indicates that the most recent heating event capable of full thermal remagnetization took place at 3.69 billion years ago (Ga) (Fig. 3C) and that the meteorite has largely escaped subsequent thermal disturbances. In particular, the observed degassing of radiogenic ^{40}Ar can be accounted for by a mean effective temperature between -50° and 140°C during the past 15 My (i.e., during transfer to Earth; see Fig. 3D). This is entirely consistent with our ^{38}Ar analysis, which reveals a cosmic ray exposure age of ~15 My, during which time the meteoroid may have been heated to a constant temperature of no more than 140°C (Fig. 3A). Blocking temperature relationships for kamacite and pyrrhotite (25, 26) and the degree of post-3.69 Ga heating inferred from ^{40}Ar and ^{38}Ar diffusion suggest that the HC/HT component should have survived from 3.69 Ga to the present day.

Our ARM and IRM paleointensity experiments indicate that the magnetizing field that produced the HC/HT component at 3.69 Ga most likely had

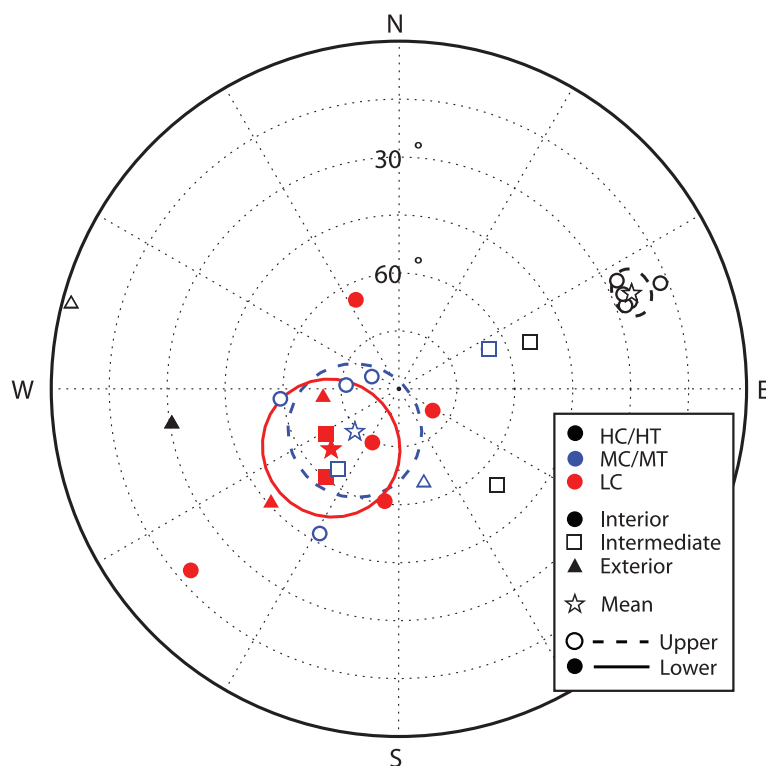


Fig. 1. NRM directions in eucrite ALHA81001. This is an equal-area stereographic projection showing the three components of magnetization observed in each subsample as inferred from principal components analyses. Black, blue, and red symbols represent HC/HT, MC/MT, and LC directions, respectively. Stars indicate average directions for each component. Dashed circles denote the 95% confidence interval for the true direction of magnetization assuming a Fisher distribution in the upper hemisphere, and solid circles denote it in the lower hemisphere. Exterior fusion-crust subsamples, intermediate-depth subsamples (depth from surface between 0.7 and 2.0 mm), and interior subsamples (depth >2 mm) are shown by triangles, squares, and circles, respectively. Open symbols represent the upper hemisphere; solid symbols represent the lower hemisphere.

an intensity of $\sim 12 \mu\text{T}$ (with a minimum value of $\sim 2 \mu\text{T}$) (27). The young $^{40}\text{Ar}/^{39}\text{Ar}$ age of ALHA81001 precludes the direct recording of a dynamo, because the longest predicted duration of a dynamo for a Vesta-sized object is on the order of several tens of millions of years to ~ 100 My after solar system formation (28, 29). Likewise, solar and nebular fields could not be a source of the magnetization, because they should have dissipated within the first ~ 6 My of solar system formation (30). Furthermore, slow cooling of ALHA81001 over $>10^2$ years rules out the recording of any putative transient, impact-generated fields, which are expected to have persisted for less than several hundred seconds under Vestan impact conditions (31). This leaves remanent magnetization of the Vestan crust and underlying materials as the most likely magnetic field source. This in turn requires that the crust was magnetized by an earlier ambient magnetic field. Given the high inferred paleointensities for ALHA81001, this earlier magnetic field was most likely due to a core dynamo. Although nebular fields may have had intensities comparable to those of core dynamos (32), they should have existed only during the first <6 My after solar system formation and typically varied in direction on time scales of several tens of orbits. Petrographic studies show that Vesta's crust cooled from the 780°C Curie point of kamacite to ambient space temperatures over at least several million years and therefore is unlikely to have coherently recorded such time-variable nebular fields (33). Another possibility is that Vesta's crust near or antipodal to large impact basins may have acquired remanent magnetization due to transient, impact-generated magnetic fields (34, 35). However, the low velocities (~ 5 km/s) expected for impacts on Vesta should not have typically produced the ionized plasma clouds necessary for generating strong transient magnetic fields (36–38). On the other hand, an early dynamo field should have been capable of generating steady core magnetic fields with intensities up to $2600 \mu\text{T}$, resulting in surface fields of up to the order of $100 \mu\text{T}$ assuming a dominantly dipolar field geometry (13, 39), thereby providing a means of magnetizing the Vestan crust and underlying material.

Following (40), we calculated the expected strength of the remanent crustal field due to this earlier dynamo. Although most HED meteorites exhibit saturation remanence of between 10^{-4} and $10^{-2} \text{ A}\cdot\text{m}^2 \text{ kg}^{-1}$ (41), certain previously unmeasured metal-rich HEDs may have higher values. We measured the saturation remanence of one such HED meteorite, Camel Donga, to be $5 \times 10^{-2} \text{ A}\cdot\text{m}^2 \text{ kg}^{-1}$ (31). Using this value for saturation remanence, a $100\text{-}\mu\text{T}$ field is expected to impart a TRM of intensity M_{TRM} up to 3×10^{-6} to $2 \times 10^{-3} \text{ A}\cdot\text{m}^2 \text{ kg}^{-1}$ in HED material (42). The resulting magnetic fields generated by a crust magnetized to these values depend on the specific geometry of the magnetized material. We calculated the expected field for one plausible geometry. Because ALHA81001 is probably an

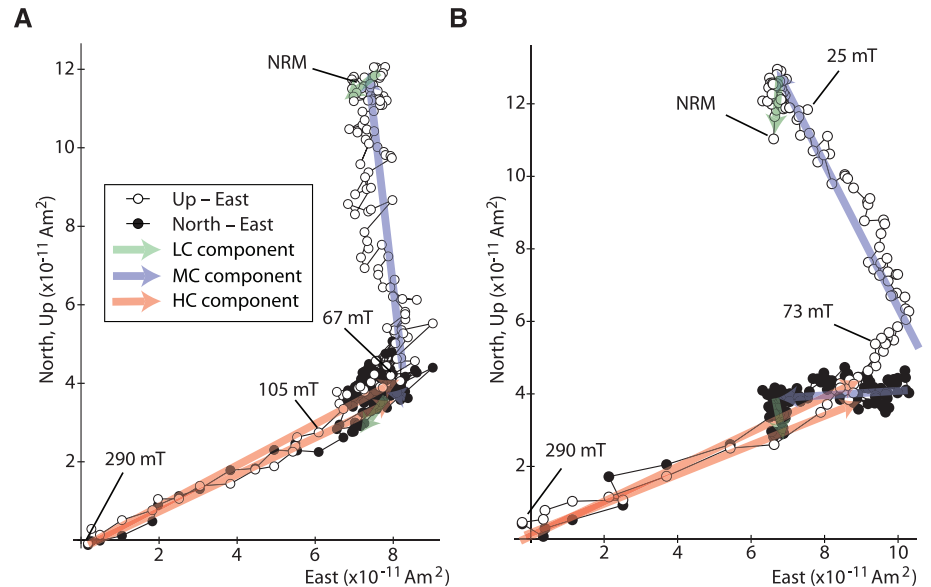


Fig. 2. Demagnetization of eucrite ALHA81001. Orthographic projections of the evolution of the NRM during AF demagnetization of two interior subsamples are shown. Open and solid circles indicate the projection of the NRM vector onto the vertical (up-east) and horizontal (north-east) planes, respectively. Red, blue, and green arrows denote HC, MC, and LC components, respectively. Selected AF levels are labeled. (A) AF demagnetization of interior subsample A5. (B) AF demagnetization of interior subsample A6. The similarity between the two demagnetization sequences indicates unidirectional magnetization.

impact melt (43), we evaluated the magnetic field within an impact-heated region in a thin crust magnetized perpendicular to the plane (31). The resulting field is $\sim 2/3 \mu_0 M_{\text{TRM}}/\rho$, where μ_0 is the permeability of free space and ρ is the crustal density, assumed to be 3000 kg m^{-3} . Crustal material on Vesta magnetized in a dynamo with this geometry can therefore generate magnetic field intensities between 0.01 and $\sim 4 \mu\text{T}$.

Although the upper end of this range agrees with our inferred paleointensities, our paleointensities are nevertheless surprisingly high and may suggest the presence of more strongly magnetic material on or beneath the surface of Vesta that is not sampled by HED meteorites. Carbonaceous chondrite material has been observed in howardites and may have been observed as localized dark terrains on the surface of Vesta (44). Mesosiderites have been hypothesized to originate on Vesta because of the similarity of their oxygen isotopic compositions to those of HEDs (45). Carbonaceous chondrites and mesosiderites have saturation remanence values in excess of $10^{-1} \text{ A}\cdot\text{m}^2 \text{ kg}^{-1}$ (41) and therefore can readily produce $>10\text{-}\mu\text{T}$ crustal magnetic fields when magnetized in a $100\text{-}\mu\text{T}$ dynamo field. Therefore, such material, if present on Vesta, may result in localized, highly magnetic terrains. The high paleointensity of ALHA81001, which is stronger than that of most previously studied HEDs (10), may attest to the relative rarity of the purported highly magnetic terrains. Localized regions of high crustal field intensities on Mars and the Moon are similarly too intense to be explained by the observed magnetism of known martian meteorites

and Apollo samples, respectively, and also require the existence of unsampled, highly magnetic material on these bodies (40, 46). A magnetized crust due to a prior dynamo epoch therefore appears to be the only plausible field-generation mechanism consistent with our inferred paleointensities.

Our inferred detection of an early dynamo in Vesta provides further evidence that dynamos could have formed in small differentiated bodies in the early solar system. The presence of a magnetized crust implies that at least parts of the Vestan surface had solidified and cooled below the Curie temperature during the presence of an active dynamo. The strong inferred intensity of remanent crustal magnetization suggests that the dynamo, when it was active, probably generated surface fields with intensities between 10 and $100 \mu\text{T}$ and that strongly magnetic material unsampled by HED meteorites may be present on or below the surface of Vesta. Furthermore, the existence of crustal magnetization at 3.69 Ga suggests that Vesta is likely to have crustal magnetic fields at the present time, because impact events are unlikely to have demagnetized the entire surface. Spectral features of the Vestan surface indicate that space weathering effects are more subdued than those observed on the Moon (47) and may require shielding from the solar wind ion flux at 2.36 astronomical units by a surface field $\geq 0.2 \mu\text{T}$ (48). The existence of $>2\text{-}\mu\text{T}$ magnetic fields of the intensities estimated here are therefore sufficient to stand off solar wind ions at the orbital distance of Vesta, providing a possible explanation for the apparently limited effects of solar wind ion-generated space weathering.

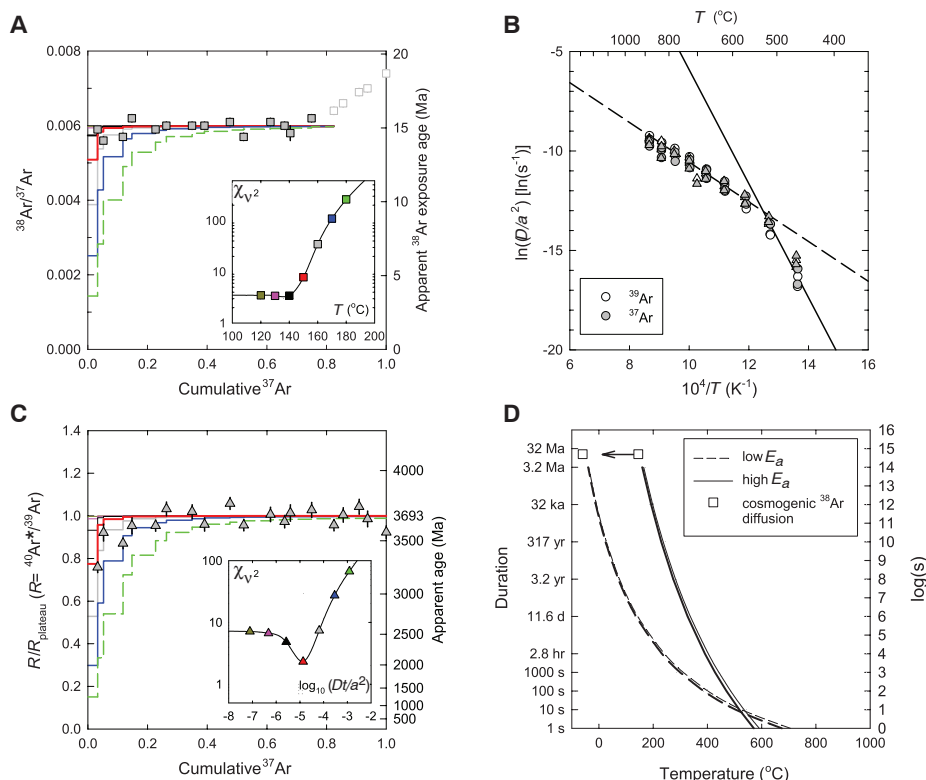


Fig. 3. ^{40}Ar and ^{38}Ar thermochronometry of ALHA81001. **(A)** Cosmogenic ^{38}Ar . Squares are observed $^{38}\text{Ar}/^{37}\text{Ar}$ ratios ± 1 SD versus the release fraction of ^{37}Ar . Colored steps are synthetic release spectra calculated for the production and diffusion of cosmogenic ^{38}Ar over the apparent exposure duration of 15 My, assuming the Ar diffusion kinetics with maximum activation energy (E_a) in **(B)** (solid line) and isothermal temperatures ranging from $<120^\circ$ to 180°C . The inset shows reduced chi-squared (χ^2) fit statistics of each model compared to solid points, identifying the best-fit temperatures to be $\leq 140^\circ\text{C}$. **(B)** Diffusivity as a function of temperature (T) calculated (49) from ^{37}Ar and ^{39}Ar released during the first 19 heating steps of two degassing experiments; points are diffusion coefficients (D) divided by the square of the effective domain radius (a). The solid line quantifies kinetics with the apparent E_a determined from regression to the initial four extractions of each experiment; the dashed line gives the apparent E_a determined from the subsequent 16 extractions. The difference in these values of E_a may be due to a change in a or a non-uniform spatial distribution of ^{37}Ar and ^{39}Ar (43). We take the values of E_a for the first four extractions and the subsequent extractions as upper and lower bounds on the true value. **(C)** Production and diffusion of radiogenic Ar ($^{40}\text{Ar}^*$). Triangles are measured $^{40}\text{Ar}^*/^{39}\text{Ar}$ ratios (R) normalized to the mean ratio of the apparent plateau (R_{plateau}) versus the cumulative ^{37}Ar release fractions. The colored steps are model release spectra for heating conditions, as in **(A)**, normalized to a mean plateau age of 3.693 ± 0.071 Ga (± 1 SD); the inset identifies $\log_{10}(Dt/a^2) = -5$ as the best-fit solution, where t is the duration of heating. **(D)** Duration and temperature constraints on possible thermal excursions experienced by ALHA81001. Solid curves are upper bounds on permissible thermal events at 15 million years ago (Ma) (bold curve) and at 1.0 Ga (thin curve) that would best predict the observed $^{40}\text{Ar}^*/^{39}\text{Ar}$ spectrum shown in **(C)**, using the maximum apparent E_a in **(B)**; dashed curves are calculated from models using the minimum apparent E_a in **(B)** for thermal events at 15 Ma (bold curve) and 1.0 Ga (thin curve). The squares are upper bounds from **(A)** for maximum and minimum values of E_a . Solar heating since ~ 15 Ma to mean effective temperatures between -50° and 140°C provides an internally consistent prediction of the entire Ar data set.

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Acknowledgments: We thank the Johnson Space Center staff and the Meteorite Working Group for allocating ALHA81001, A. Bevan at the Western Australia Museum for allocating Millbillillie, P. Rochette for helpful discussions, N. Chatterjee for help with the microprobe analyses, S. Chu for help with the MPMS-XL analyses, and B. Carbone for administrative help. D.L.S. thanks W. S. Cassata for helpful discussions and the Ann and Gordon Getty Foundation for support. B.P.W. thanks the NASA Origins Program and Thomas F. Peterson for support. R.R.F. thanks the NSF Graduate Research Fellowship program for support. B.P.W. and C.S. thank the NASA Lunar Science Institute for support. Paleomagnetic and thermochronology data are found in the supplementary materials.

Supplementary Materials

www.sciencemag.org/cgi/content/full/338/6104/238/DC1
Supplementary Text
Figs. S1 to S19
Tables S1 to S7
References (50–121)
Database S1

5 June 2012; accepted 12 September 2012
10.1126/science.1225648

Elemental Mapping by Dawn Reveals Exogenic H in Vesta's Regolith

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Using Dawn's Gamma Ray and Neutron Detector, we tested models of Vesta's evolution based on studies of howardite, eucrite, and diogenite (HED) meteorites. Global Fe/O and Fe/Si ratios are consistent with HED compositions. Neutron measurements confirm that a thick, diogenitic lower crust is exposed in the Rheasilvia basin, which is consistent with global magmatic differentiation. Vesta's regolith contains substantial amounts of hydrogen. The highest hydrogen concentrations coincide with older, low-albedo regions near the equator, where water ice is unstable. The young, Rheasilvia basin contains the lowest concentrations. These observations are consistent with gradual accumulation of hydrogen by infall of carbonaceous chondrites—observed as clasts in some howardites—and subsequent removal or burial of this material by large impacts.

Spectra of asteroid 4 Vesta are characterized by strong absorption bands of FeO-bearing pyroxenes that are indistinguishable from spectra of howardite, eucrite, and diogenite (HED) meteorites (1). Identification of a family of small Vesta-like asteroids (vestoids) derived from Vesta has led to the consensus that Vesta is the HED parent asteroid (2). Studies of HEDs indicate that Vesta is differentiated (3–6). The canonical model for the petrologic evolution of Vesta based on HEDs indicates that Vesta was substantially melted within a very few million years of the solar system's formation, forming a molten core overlain by a shell of partially or totally molten silicates (3, 5, 6). Crystallization of a global silicate magma ocean produced an olivine-dominated mantle, a lower crust rich in low-Ca pyroxene ± olivine (diogenite), and an upper crust of mafic flows and intrusions (eucrite) (3, 5, 6). Alternatively, serial magmatism could have emplaced diogenite plutons within the lower crust or at the crust-mantle bound-

ary (5). Impact excavation and mixing produced a polymict regolith with a varying diogenite-to-eucrite ratio, which produces howardites when lithified by impact (3, 5–7). Modeling of regolith formation on 100- to 300-km-diameter asteroids shows the howarditic debris layer should be hundreds of meters thick (8).

Dawn's Gamma Ray and Neutron Detector (GRaND) is used to measure gamma rays and neutrons produced by cosmogenic nuclear reactions and radioactive decay to depths of a few decimeters within Vesta's surface (9). Concentrations and detection limits for several elements and constraints on elemental abundances, including effective atomic mass and neutron absorption, can be determined. With these measurements, GRaND allows us to distinguish between the whole-rock compositions of HED end-members (10) and other lithologies that may be present (9). Gamma ray and neutron mapping data were acquired over almost 5 months in a circular, polar

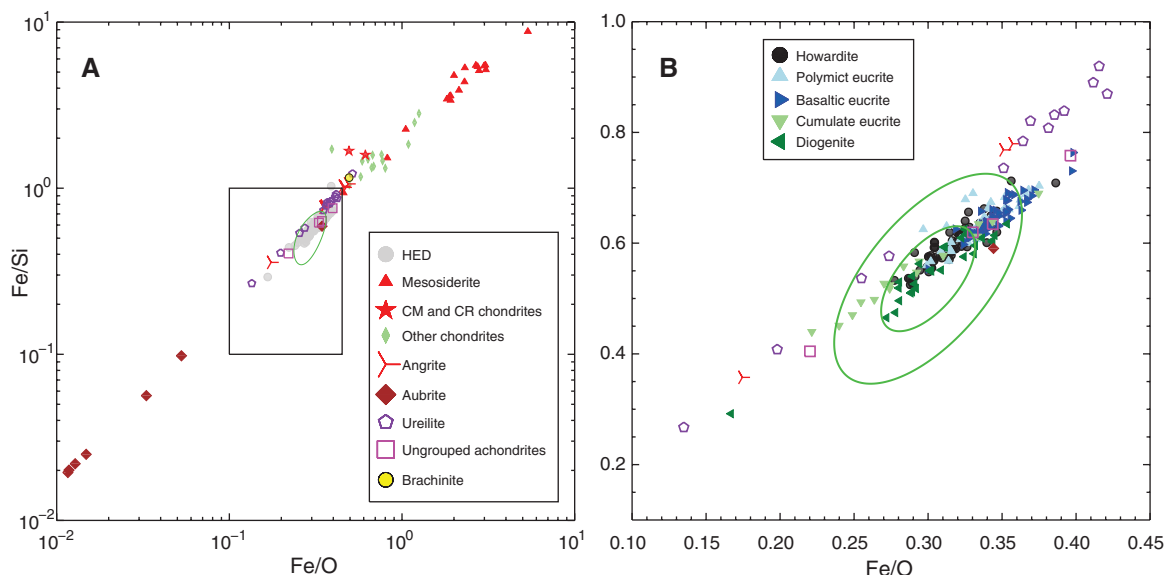
low-altitude mapping orbit (LAMO) with an average altitude of 210 km (11, 12). Here, we compare these direct measurements of the elemental composition of Vesta's regolith with HED meteorites.

The gamma-ray spectra acquired by the bismuth-germanate (BGO) scintillator enables the determination of global Fe/O and Si/O mass ratios (12). Our conservative estimate for Fe/O is 0.30 ± 0.04 and that for Si/O is 0.56 ± 0.06 , giving an Fe/Si ratio of 0.54 ± 0.09 . The different achondrite, chondrite, and stony iron meteorite groups have wide ranges in Fe/O and Fe/Si, with the aubrites, all chondrites, and mesosiderites being quite distinct from HEDs (Fig. 1A). Some angrites, ureilites, and the anomalous aubrite, Shallowater, overlap HED compositions on this plot (Fig. 1B), but their visible and infrared spectra are easily distinguishable from those of HEDs (13–15). Some ungrouped basaltic achondrites also fall within the field of HEDs. Mineralogically and compositionally, these are very similar to basaltic eucrites and would be difficult to distinguish from the latter with Dawn instrumentation (16). None of the ungrouped basaltic achondrites are regolith breccias. Thus, the characteristics of the debris layers on their parent asteroids are unknown.

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Fig. 1. Plots of Fe/Si versus Fe/O (by mass) for various achondrites, chondrites, and the stony iron mesosiderites are compared with HED meteorites. Box in (A) shows region expanded in (B). Error ellipses (1 σ and 2 σ) of the GRaND data are shown.



The GRaND error ellipse for the Fe/O and Fe/Si ratios includes howardites (Fig. 1B) but is skewed toward greater proportions of diogenite and cumulate eucrite (lower crustal materials) than basaltic eucrite. The Rheasilvia impact excavated and globally distributed lower crustal materials (17, 18) and formed the family of vestoids (19). Howardites are likely derived from the vestoids, and thus represent Vesta's more basaltic-rich regolith before deposition of lower crustal materials ejected by the large, Rheasilvia impact. Never-

theless, the near equivalence in GRaND compositional data and howardites (Fig. 1) strengthens the Vesta-HED link and indicates that the vestan surface is consistent with eucrite and diogenite mixtures (12).

GRaND is sensitive to neutrons with kinetic energies within three ranges: fast (>0.7 MeV); epithermal (0.1 eV to 0.7 MeV); and thermal (<0.1 eV) (9, 12). Neutron counting rates presented here were measured with a lithium-loaded glass (LiG) scintillator, which is sensitive to a mix-

ture of thermal and epithermal neutrons (TPEs), and a boron-loaded plastic (BLP) scintillator, which separately measures epithermal and fast neutrons (12). Epithermal neutrons were also measured with a BLP-BGO coincidence signature (12).

Epithermal neutron counting rates (BLP_e), corrected for solid angle and variations in cosmic ray flux, depend on the abundance of H in Vesta's regolith and are relatively insensitive to other variations in composition for HED-like materials (9, 20). For a homogeneous regolith, the abundance of H (micrograms per gram) is given by

$$[H] = k[C_0/C - 1] \quad (1)$$

where $k = 2100$, C is the corrected epithermal neutron counting rate, and C_0 is the counting rate for H-free materials (12, 20). Zonally averaged neutron counting rates (Fig. 2) are systematically higher in the southern hemisphere. Assuming the highest counting rate corresponds to $[H] = 0 \mu\text{g/g}$, the 12% variation in epithermal counting rate implies a maximum zonally averaged $[H]$ of $250 \mu\text{g/g}$ near the equator. Similarly, if H was the only compositional parameter then the variations in thermal plus epithermal (TPE) and (BLP_f) fast neutron counting rates would imply a maximum of $470 \mu\text{g/g}$ H ($k = 8400$) and $840 \mu\text{g/g}$ H ($k = 11200$), respectively.

These divergent estimates of $[H]$ show that variations in the abundance of other elements affect the TPE and fast neutron measurements, which is not surprising given the variability of counting rates expected for H-free, whole-rock HED meteorite compositions (comparisons provided in Fig. 2). In contrast, the full-range variation of epithermal neutron counting rates (12%) is much larger than expected from HEDs (4%) and similar to that observed on the Moon by Lunar Prospector (11%) (20). Lunar $[H]$ varies from about $50 \mu\text{g/g}$ from solar wind-implanted protons at equatorial latitudes to hundreds of micrograms per gram in permanently shadowed craters near the poles, which are thought to contain >1% g/g water ice (20, 21).

Corrected BLP_e and TPE neutron counting rates mapped on 15° quasi-equal-area pixels show that the highest counting rates are contained within and centered on the Rheasilvia basin (Fig. 3). This observation is broadly consistent with instrument spatial-mixing of a compositionally uniform basin with surrounding regions. The lowest counting rates are at equatorial latitudes from about 60°E to 225°E . The high degree of correlation between the BLP_e and TPE neutron counting rates suggest that both are strongly influenced by variations in $[H]$.

A scatter plot of the BLP_e and TPE neutron counting rates separates contributions from H and other elements (Fig. 4). The BLP_e neutron counting rate is relatively insensitive to changes in H-free composition, whereas the TPE neutron counting rate is strongly sensitive to variations in the absorption of neutrons by the regolith. For HEDs, variations in neutron absorption are caused

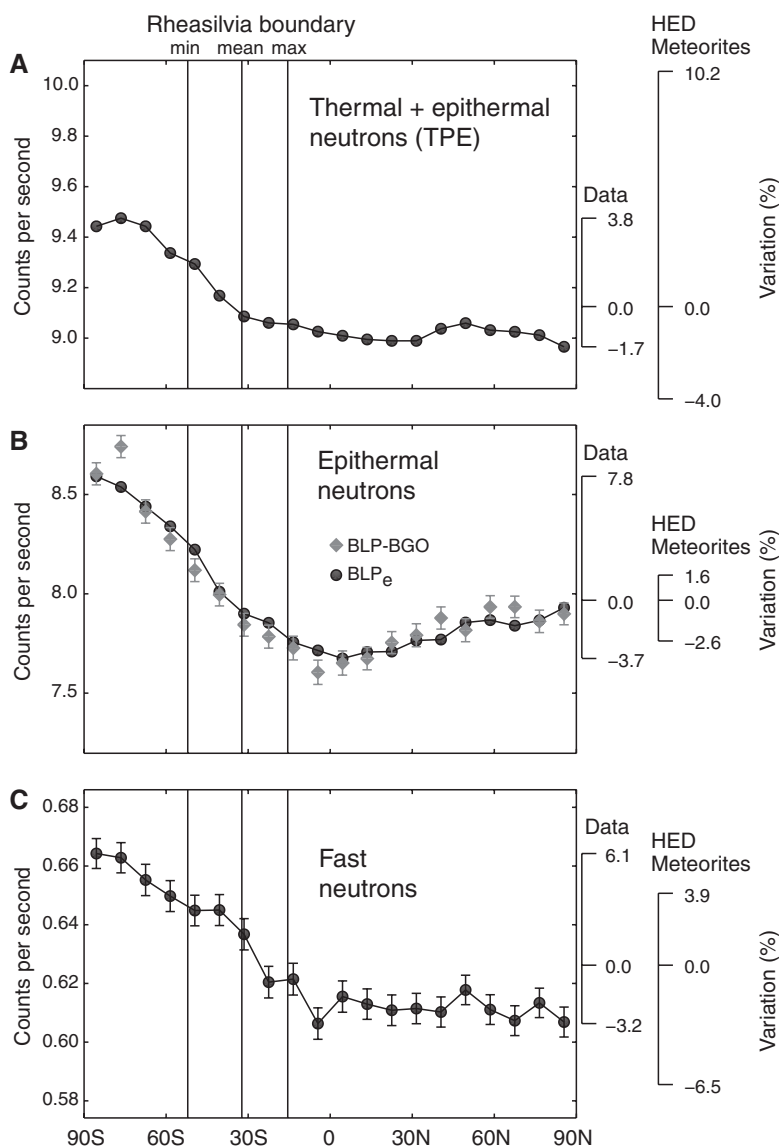


Fig. 2. Corrected neutron counting rates, binned by latitude vary with surface elemental composition. **(A)** Neutrons in the thermal + epithermal range (TPE) are sensitive to $[H]$ and thermal neutron absorption (for example, by Fe). **(B)** Neutrons in the epithermal range are primarily sensitive to variations in $[H]$. Independent measurements of the epithermal neutrons, BLP_e and BLP-BGO (9, 12), are shown. **(C)** Fast neutron measurements (BLP_f) are sensitive to variations in $[H]$ and the average atomic mass of the regolith. All counting rates are elevated in the southern hemisphere, which contains the Rheasilvia impact basin (minimum, maximum, and mean latitudes for the basin are shown). In (A) to (C), the full-range variation in the counting data are compared with the range expected for HED meteorites, determined by modeling the response of GRaND to vestan neutrons for representative, H-free, whole-rock HED compositions (10, 12). Average uncertainties for zonal TPE and BLP_e counting rates are 0.14 and 0.20%, respectively.

primarily by changes in the abundance of Fe, Ca, Al, Ti, and Mg (9). In the absence of H, diogenites, with relatively low Fe, Ca, and Al abundances, produce higher TPE neutron counting rates than that of basaltic eucrites, which have higher abundances of these elements.

For any H-free composition, increasing [H] causes the TPE and BLP_e counting rates to decrease in a predictable way (Fig. 4, red trend lines). GRaND data do not follow a single H trend line, indicating that neutron absorption is not uniform on Vesta's surface. Points within the Rheasilvia basin cluster to the right of the howardite trend line in Fig. 4, toward cumulate eucrites and diogenites. This is consistent with measurements of pyroxene band centers by Dawn's Visible-Infrared mapping spectrometer (VIR) (22), which show that Rheasilvia is more diogenitic than is the rest of Vesta. Hubble Space Telescope observations demonstrated the presence of the Rheasilvia basin, which was inferred to have excavated the lower crust or upper mantle (23); however, the multi-color camera data were interpreted to indicate high-Ca pyroxene and/or olivine and not the low-Ca pyroxene characteristic of diogenites detected with VIR.

Using Eq. 1 and assuming [H] = 0 μg/g for the maximum epithermal counting rate in Rheasilvia, the distribution of H on Vesta (Fig. 5) was determined from mapped epithermal neutron counting rates (Fig. 3A). This procedure gives a robust, lower bound on [H] at each map location, with uncertainties of ~50 μg/g. The global average [H] was 180 μg/g. Thus, Vesta's regolith contains at least 2.4×10^{11} kg of H within the 150 g/cm² depths sensed by GRaND. For a density like that of lunar soil, 1800 kg/m³, Vesta's regolith would contain an average of 0.3 kg/m³ of H in the approximately 80-cm-thick regolith layer sensed by GRaND. On the basis of comparison with data acquired with GRaND at Mars (12), the average [H] on Vesta could be higher: 800 μg/g. However, this estimate is very uncertain (±400 μg/g).

The map of [H] is compared with an albedo map in Fig. 5. The highest abundances of H correspond to the lowest-albedo regions. [H] and albedo are anticorrelated, with a linear Pearson correlation coefficient of -0.73 (12), suggesting that a dark, H-bearing component is mixed into the surface. Deviations from this correlation may arise from the large differences in the spatial resolution of the [H] and albedo maps and other sources of albedo variation, such as particle size or the presence of dark, H-free materials (such as impact melt or dewatered carbonaceous chondrites).

Hydrogen content is roughly associated with surface age, with the lowest [H] found in the younger Rheasilvia basin and the highest corresponding to dark, older units relatively uncontaminated with Rheasilvia ejecta (17, 24). The young crater, Marcia (190E, 10N), a comparatively high-albedo feature within the dark equatorial region, is associated with a local minimum in [H] (Fig. 5). Another local minimum in [H] extending from Rheasilvia into the northwestern part of a

basin containing Oppia is associated with relatively low crater density.

HED meteorite compositions show that Vesta accreted from volatile-poor materials and is similar to the Moon in its abundance of moderately volatile elements such as Na (25) and thus would have been very H-poor. Consequently, the H measured with GRaND does not have an endogenous source. Two exogenous sources are possible: implantation of solar wind H in regolith grains and the infall and survival of hydrous material from meteoroids.

Solar wind cannot explain the high-H contents (up to 400 μg/g) found in some areas. The lunar regolith contains far less H; 16 to 60 μg/g for soils and 11 to 116 μg/g for regolith breccias (26). Lunar soils contain 15 to 123 nmoles/g solar wind ²⁰Ne, and regolith breccias contain 56 to 335 nmoles/g (12). Kapoeta, a regolithic howardite (7), contains only 0.04 to 1.28 nmoles/g of ²⁰Ne in solar wind-dominated samples (27). Correcting the solar wind flux for heliocentric distance, the data show that Kapoeta had a shorter exposure than did lunar regolith breccias and can-

not contain more than 100 μg/g solar wind H and likely much less (12).

The infall of carbonaceous chondritic material containing OH-bearing phyllosilicates is a viable alternative (9). A weak detection of an OH absorption feature in Earth-based infrared spectral measurements has been interpreted as arising from carbonaceous chondritic debris on Vesta (28), although this detection was not confirmed by other Earth-based observations (29). Admixture of carbonaceous chondritic material is one of two hypotheses advanced to explain lower-albedo regions seen in Dawn Framing Camera images (30).

Incorporation of hydrous carbonaceous chondritic debris in the regolith can quantitatively explain the measured concentrations of H and the association of H with low-albedo material. Chondritic clasts have been found in the HEDs, mostly in howardites (12). Most clasts are CM or CR chondrites; ~80% are CM (31). Modal abundances of clasts are typically 2 to 5% by volume, but an exceptional sample contains up to 60% clasts (32). Some clasts have been partially dehydrated during impact (31). The average H

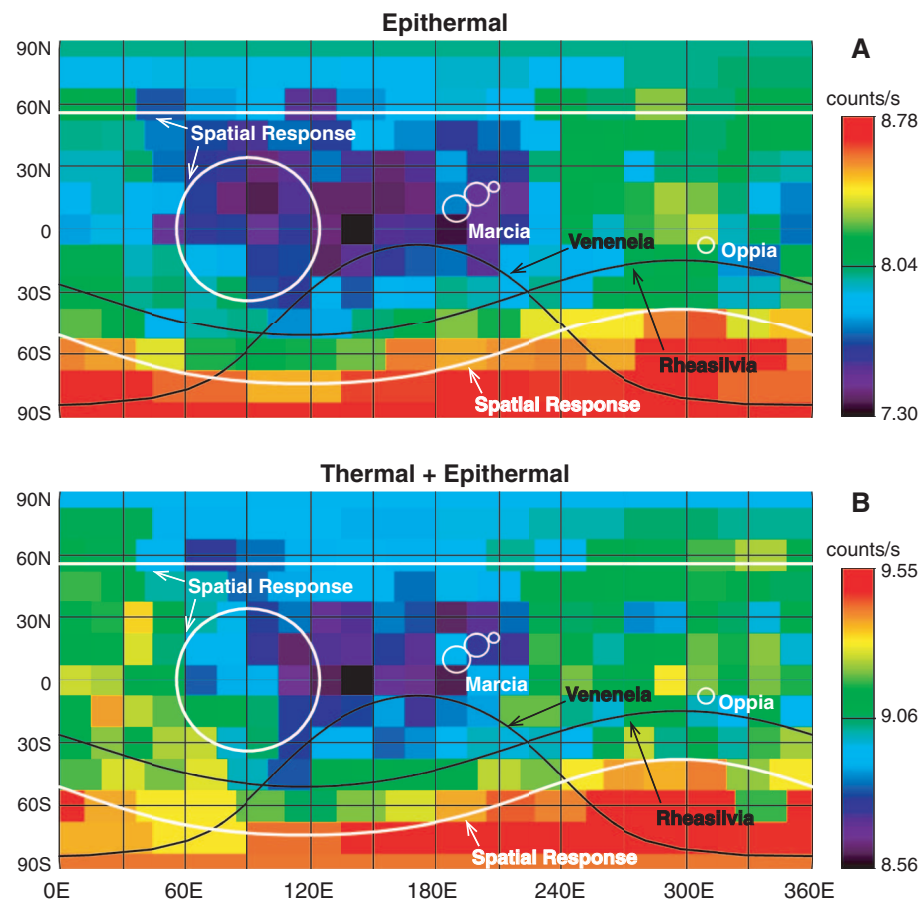


Fig. 3. Maps of corrected (A) epithermal (BLP_e) and (B) thermal + epithermal (TPE) neutron counting rates binned on 15° quasi-equal-area pixels reveal compositional variations on Vesta's surface. Longitude convention used is based on the Claudia system (11). The boundaries of two large-impact basins in the southern hemisphere are shown. Craters mentioned in the text are indicated. The spatial resolution (half-maximum) of GRaND is indicated as circles (white) for three subsatellite locations: the north pole, the equator, and at the center of Rheasilvia. Roughly half of the counts measured by GRaND at any location originate within the half-maximum. GRaND fully resolves features separated by a circle-diameter.

content of CM chondrites is 1.2 weight percent (33), and CR chondrites have comparable H contents (34). The abundances of carbonaceous

chondrite clasts translate to 240 to 600 $\mu\text{g/g}$ H for bulk howardites, assuming little dehydration has occurred, which is similar to abundances

found on Vesta. Volatilization of hydrogen-bearing materials by high-velocity impacts may have resulted in the formation of pitted terrain (35); one

Fig. 4. Contributions from H and neutron absorption are distinguished by a scatterplot of the mapped epithermal (BLP_e) and thermal + epithermal (TPE) counting rates (Fig. 3). Representative error bars are shown. The data are compared with simulated neutron counting rates for HED whole-rock compositions (12). Counting rate trends with [H] are shown for selected compositions. [H] is indicated for selected points along the Howardite + H trend line. Both the models and data were arbitrarily normalized for this comparison.

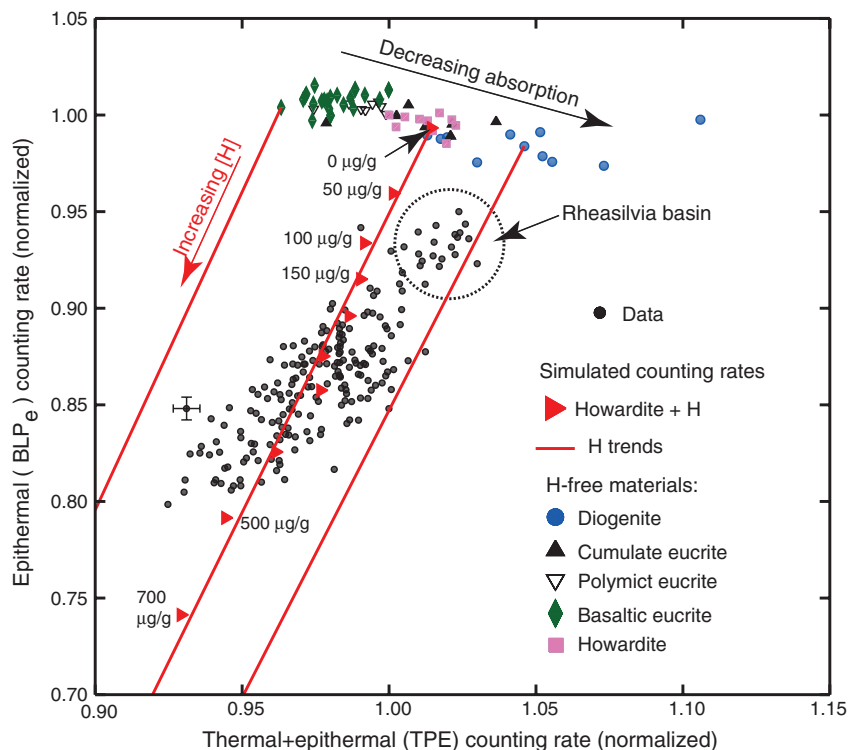
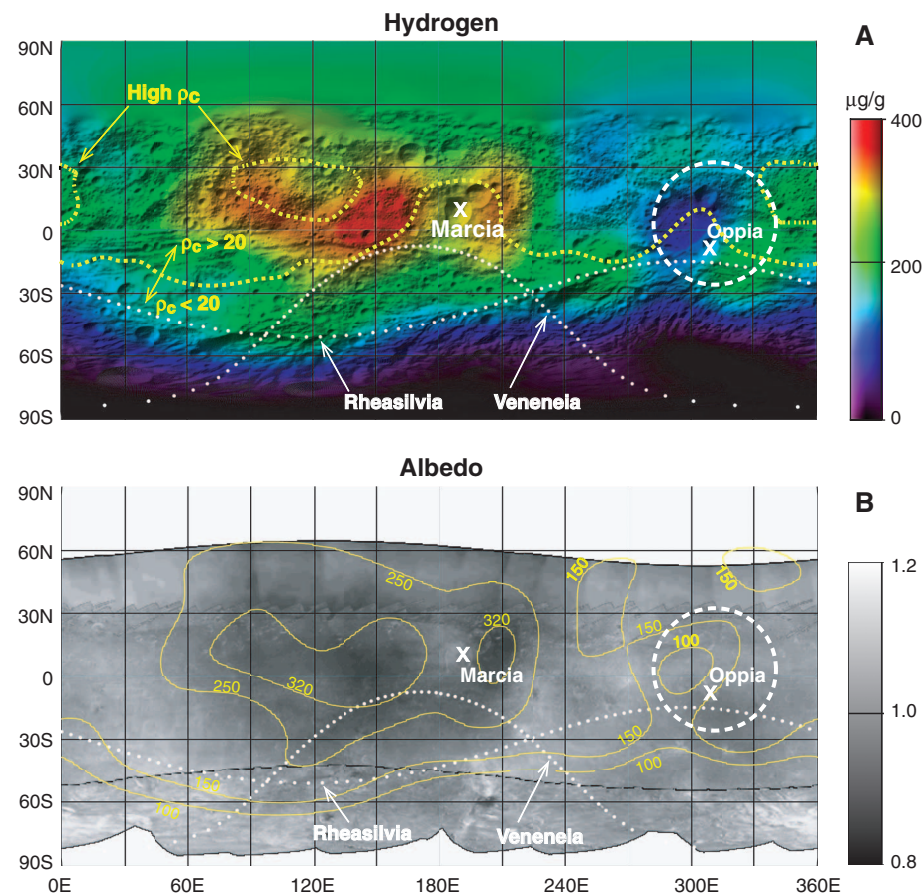


Fig. 5. A map of [H], superimposed on shaded relief (A), is compared with an albedo map (B) of Vesta (12, 30, 37). The dashed white line outlines a circular depression, containing crater Oppia (24). Approximate contours of smoothed crater density (ρ_c in craters per 10^4 km^2), superimposed in yellow on the [H] map indicate relative age of the surface (24). Yellow contours of [H], (in micrograms per gram), are superimposed on the map of albedo. Roughly 30% of Vesta's surface is contained within the 250 $\mu\text{g/g}$ and higher contours.



such terrain corresponds to the low-[H] region around Marcia crater.

The extensive region on Vesta with elevated H content is not plausibly due to a localized enhancement in meteoroid flux or a single isolated impact—for example, fragments of the Veneneia basin impactor. Regolithic howardites such as Kapoeta contain CM, CR, and CI chondrite clasts (31) in modest abundance (12), which suggests accumulation over time from numerous impactors and asteroidal dust (36). Thus, the H-rich region of Vesta probably reflects a zone of more ancient regolith, on which the accumulation of chondritic debris has had a longer history. If Rheasilvia ejecta blanketed this region, it was much thinner than elsewhere on Vesta, so that subsequent gardening has mixed in more of the underlying ancient, carbonaceous chondrite-rich regolith.

The deposition of exogenic material is time-dependent, with H accumulating gradually on exposed surfaces. Accumulation is reset by impact excavation, volatilization, and mantling by ejecta. Thus, the [H] measured with GRaND provides a measure of the relative age of the vestan regolith on a global scale.

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Acknowledgments: We thank the Dawn team for spacecraft and instrument operations at Vesta. Portions of this work were performed by the Planetary Science Institute under contract with the Jet Propulsion Laboratory (JPL), California Institute of Technology; by JPL under contract with NASA; and by the NASA Dawn Participating Scientist Program. D. Bazell and P. Peplowski of Johns Hopkins University Applied Physics Laboratory assisted in fast-neutron data analysis. The Dawn mission is led by the University of California, Los Angeles, and managed by JPL under the auspices of the NASA Discovery Program Office. The Dawn data are archived with the NASA Planetary Data System.

Supplementary Materials

www.sciencemag.org/cgi/content/full/science.1225354/DC1
Supplementary Text
Figs. S1 to S27
Tables S1 to S4
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29 May 2012; accepted 13 August 2012
Published online 20 September 2012;
10.1126/science.1225354

Pitted Terrain on Vesta and Implications for the Presence of Volatiles

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We investigated the origin of unusual pitted terrain on asteroid Vesta, revealed in images from the Dawn spacecraft. Pitted terrain is characterized by irregular rimless depressions found in and around several impact craters, with a distinct morphology not observed on other airless bodies. Similar terrain is associated with numerous martian craters, where pits are thought to form through degassing of volatile-bearing material heated by the impact. Pitted terrain on Vesta may have formed in a similar manner, which indicates that portions of the surface contain a relatively large volatile component. Exogenic materials, such as water-rich carbonaceous chondrites, may be the source of volatiles, suggesting that impactor materials are preserved locally in relatively high abundance on Vesta and that impactor composition has played an important role in shaping the asteroid's geology.

In July 2011, the Dawn spacecraft entered into orbit around Vesta, the second-most massive asteroid in the solar system. After initial Survey and High-Altitude orbits, Dawn spiraled down to its ~210-km Low-Altitude Mapping Orbit (LAMO) (1), allowing for acquisition of Framing Camera (FC) images (2) at pixel scales of <20 m, as well as high-resolution views of Vesta's geology. LAMO clear-filter images cover >70% of the surface (latitudes above ~55°N were in shadow). In this data set, we identified

terrain with a distinct pitted morphology. Here, we describe this terrain and its implications for the presence and origin of volatiles on Vesta.

The most widespread occurrence of pitted terrain is associated with Marcia crater (~70-km diameter, Fig. 1A). Marcia is among the most recent large impacts on Vesta; using the methods of Marchi et al. (3), we estimate its age to be ~70 million years. Pitted terrain is found on otherwise smooth deposits located on the crater floor surrounding a small central peak, atop a slump ter-

race, and within portions of the continuous ejecta blanket. Pits lack raised rims, and on the floor they range in size from ~30 m (near the limit of resolution at 17 m per pixel) to just over 1 km in diameter (Fig. 1, B to D). Pits found in clusters on the slump terrace and ejecta blanket are smaller (largest sizes: ~250 m) and are often located where ejecta fills topographic lows (Fig. 1, A and F). On the floor, material that slumped down the crater walls appears to bury the pitted terrain in several areas; in others, pits may occur within the slump deposits (fig. S1). Toward the center of the floor where the deposit is probably thickest, pits increase in size, and their shapes become more

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irregular (Fig. 1, C and D). In many places, pits coalesce and overlap, resulting in polygonal boundaries between adjacent pits. A digital terrain model (4) derived from LAMO images shows that individual pits are typically <50 m deep (Fig. 1B); the largest pit complex is ~200 m deep.

The floor deposit in which Marcia's pitted terrain resides is relatively flat, apart from a broad region in the southeast that is ~200 m below the rest of the crater floor (Fig. 1B). This region contains both pits and relatively smooth areas; if the crater floor once approximated an equipotential surface, this implies a high degree of subsidence. Around the margins of the broad depression are several slump scarps (Fig. 1E), and we see evidence for subsidence at the edge of a complex pit (Fig. 1D) and surrounding a large isolated pit, where several terraces may indicate successive levels of downslope movement (Fig. 1C).

Pits in the fresh crater Comelia (15-km diameter, Fig. 2A) have similar morphologies but are restricted to the crater floor (Fig. 2B) and are smaller (<350 m). In some areas, pits abut or

form within slumped material (Fig. 2B and fig. S1). Within Licinia crater (24-km diameter, Fig. 2C), the identification of pitted terrain is tentative. The occurrence is limited, with small clusters of pits surrounded by larger expanses of smooth floor material (Fig. 2D). Licinia is slightly more degraded with more superposed impact craters, and pits are not as sharply defined. Pit sizes range from ~30 to 500 m. The floor of Numisia crater (33-km diameter, Fig. 2E) is even more ambiguous, with a small hummocky area that could represent a degraded cluster of pits that are each less than ~600 m across (Fig. 2F). The positive identification of pitted terrain within two of the youngest craters on Vesta suggests that its occurrence may have been more widespread but has degraded or been buried with time.

Marcia, Comelia, and Numisia craters all have large exposures of dark material (5–7); Licinia has not been observed at illumination conditions favorable for the assessment of albedo. FC color images and spectra from the Visible and Infrared Spectrometer (VIR) (8) show that the floor

deposits at Marcia and Comelia are distinct in color from their surroundings (Fig. 3), with 6 to 13% lower reflectance at 750 nm than average values for Vesta, whereas the dark material at each crater is 35 to 39% lower in reflectance. Pyroxene absorption bands are 4 to 9% shallower in the floor deposit than average for Vesta; local dark material has 15 to 21% shallower bands. VIR emission spectra show that the floor deposits have distinct thermal properties (fig. S2).

Analogous pitted terrain is not observed on other airless bodies (fig. S3), and the lack of alignment and distinct morphology are inconsistent with drainage of material into subsurface fractures (fig. S4). Pitted terrain in more than 200 fresh craters on Mars has similar morphologies (Fig. 4) and corresponding occurrences on floor deposits, terraces, and ejecta (9–13). Formation models for the martian pitted terrain include collapse due to sublimation of ice long after the impact event (11) or erosion due to rapid degassing of volatiles within a melt-breccia mixture heated by the impact event (12, 13). Either scenario

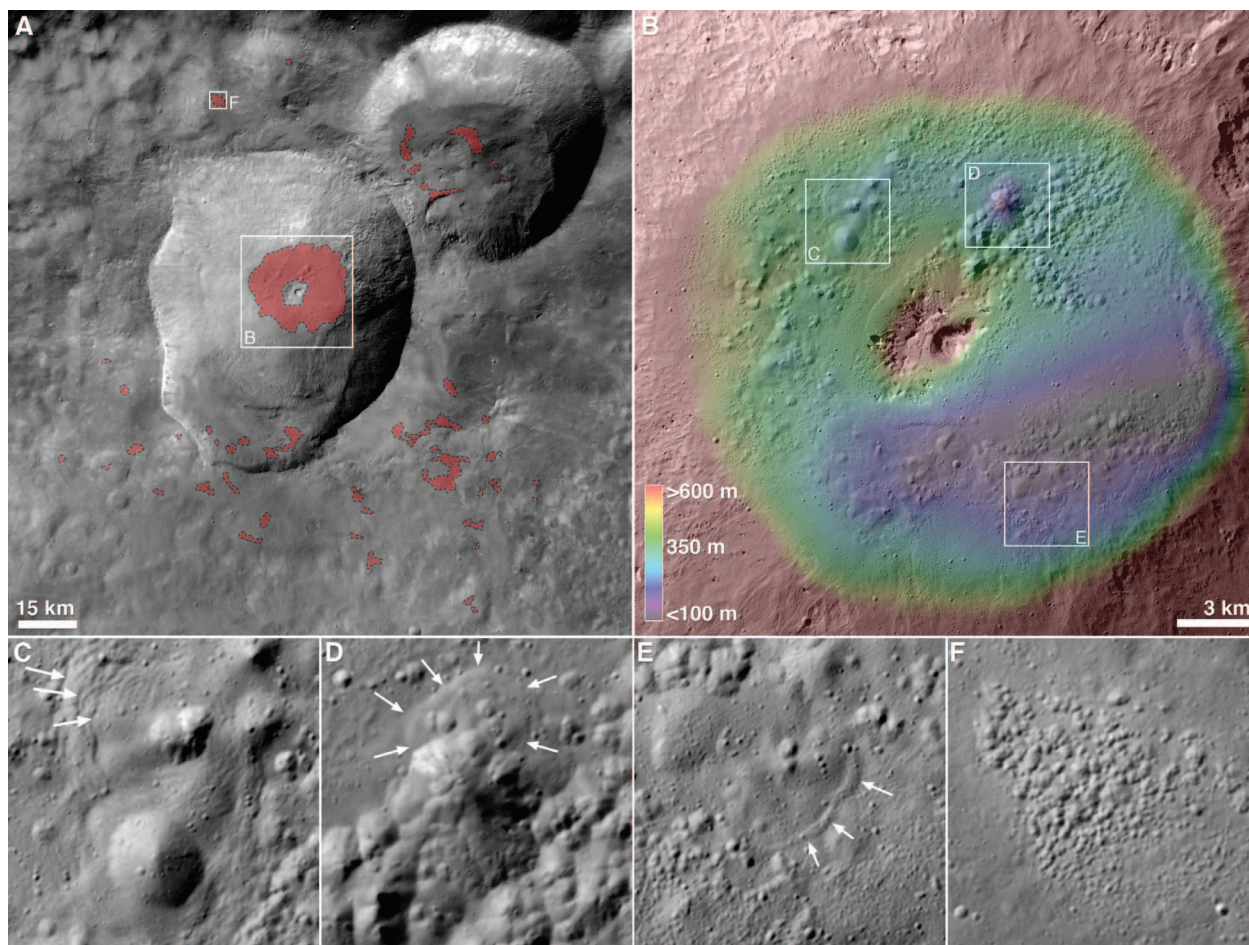


Fig. 1. Clear filter mosaic of pitted terrain at Marcia crater. (A) The sharp, raised rim indicates that Marcia is one of the youngest craters on Vesta. Locations of pitted terrain are indicated in red (boundaries approximate). North is toward the top and illumination is from the east in all images. (B) The crater floor displays the largest concentration of pits. A colorized digital terrain model derived with elevations calculated relative to a geoid is shown overlaid on a LAMO image mosaic. (C) Pits on the floor display successive levels of downslope movement.

The top, irregular pit has ripplelike features that may also be constructional [width of (C) to (F) is 3.5 km; locations of (C) to (E) are shown in (B)]. (D) Complex pitted terrain where pits share walls in near-polygonal boundaries and appear to coalesce. Arrows indicate a region of subsidence surrounding and overlapping several pits. (E) Slump scarp that probably indicates downslope movement due to subsidence. (F) A cluster of pits within the ejecta of Marcia [location shown in (A)]. All images were photometrically corrected using the procedure of Reddy *et al.* (7).

requires volatiles, presumed to be largely water ice, in substantial abundances [minimum abundance is not well defined; estimates range up to 12 weight percent (wt %) (12)].

The marked similarities between pitted terrains on Mars and Vesta suggest a similar origin. However, the prospect of abundant volatiles originating on Vesta is counter to the generally low endogenic volatile contents (14) indicated by meteorites thought to originate from Vesta (15, 16), and Vesta's basaltic crust is thought to have

degassed (17). Thus, the source of volatiles is likely to be exogenic. The association of pitted terrain with craters that have prominent exposures of dark material within their walls and ejecta (Fig. 3) may be a key observation. Telescopic spectra indicate the presence of OH and possibly H₂O on Vesta (18, 19); Dawn's Gamma Ray and Neutron Detector (GRaND) observes a heterogeneous distribution of H across the surface and finds that the highest abundances are associated with broad regions of low albedo (20). Carbonaceous chon-

drites, which have been observed as clasts in howardites (21, 22), are both low in reflectance (23) and contain an average of 9 wt % mineralogically bound water (24). The spectral properties of dark deposits indicate that carbonaceous material may comprise up to 60% of the regolith (25), which would indicate that ~5 wt % H₂O may be present in these areas. Later impacts into this water-bearing regolith would result in devolatilization due to impact heating and melting. Evidence for impact melt is seen at both Marcia and Comelia (fig. S5),

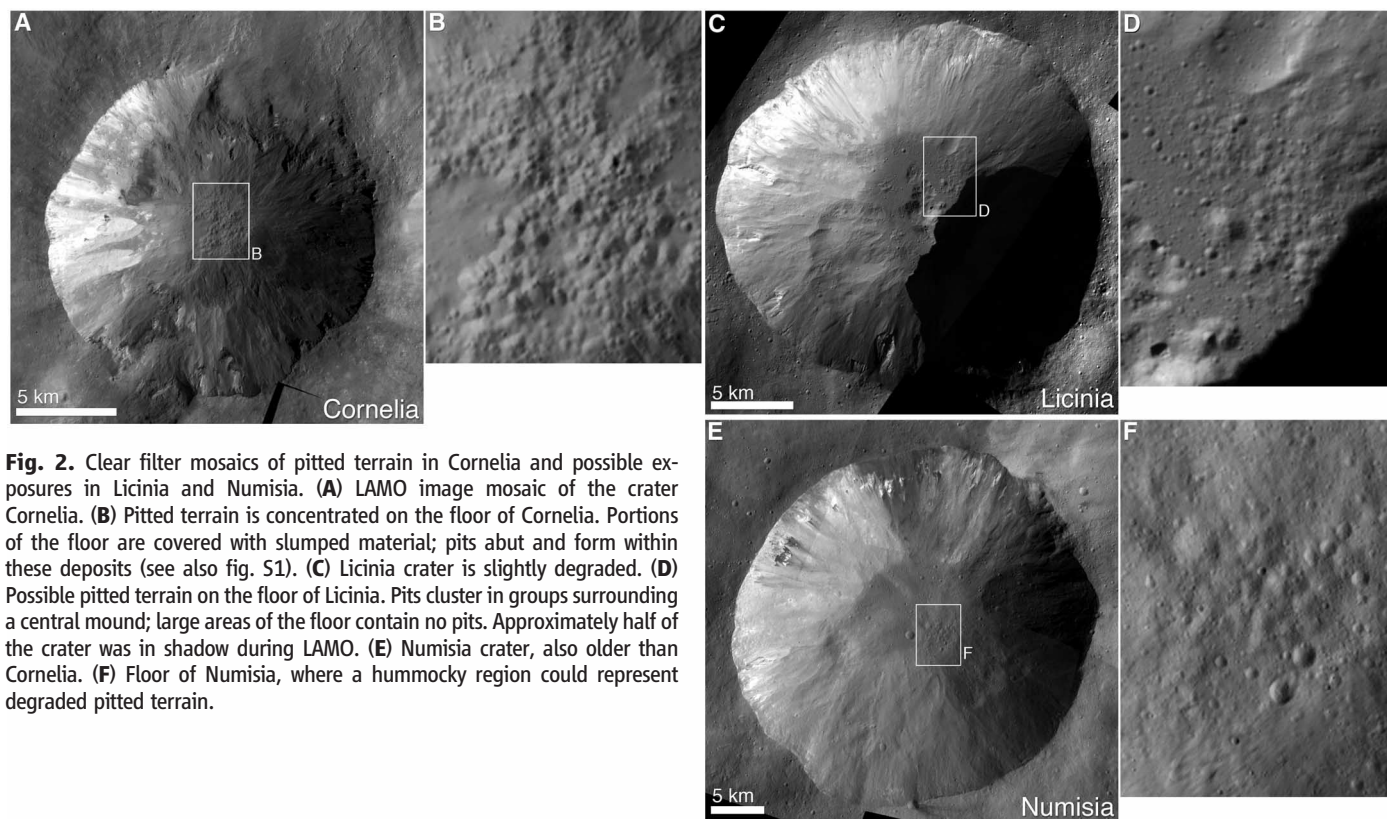


Fig. 2. Clear filter mosaics of pitted terrain in Cornelia and possible exposures in Licinia and Numisia. (A) LAMO image mosaic of the crater Cornelia. (B) Pitted terrain is concentrated on the floor of Cornelia. Portions of the floor are covered with slumped material; pits abut and form within these deposits (see also fig. S1). (C) Licinia crater is slightly degraded. (D) Possible pitted terrain on the floor of Licinia. Pits cluster in groups surrounding a central mound; large areas of the floor contain no pits. Approximately half of the crater was in shadow during LAMO. (E) Numisia crater, also older than Cornelia. (F) Floor of Numisia, where a hummocky region could represent degraded pitted terrain.

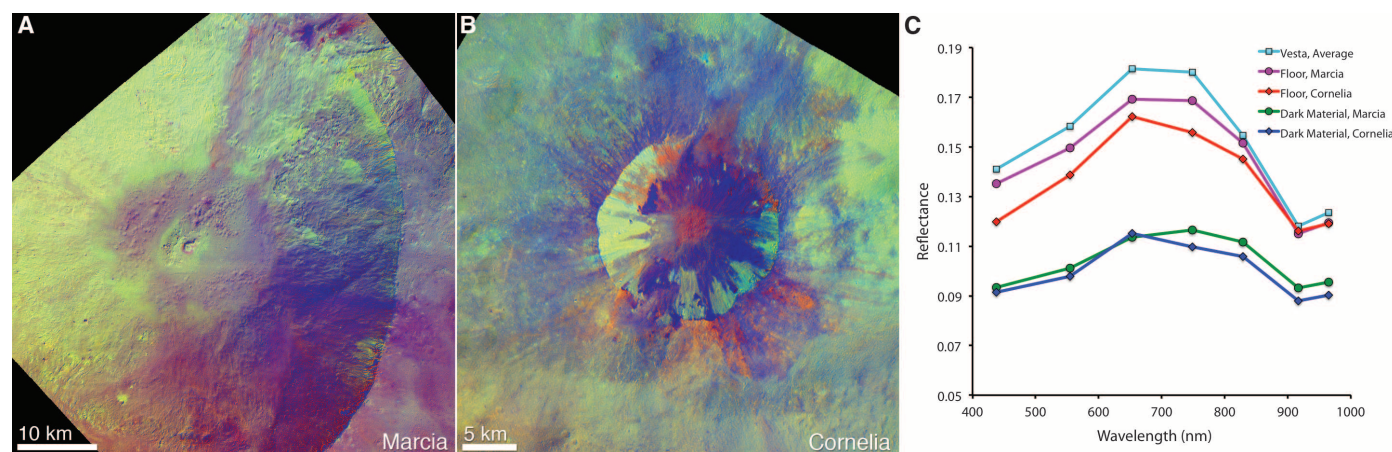


Fig. 3. Spectral properties of Marcia and Cornelia craters. (A) Enhanced color view of Marcia crater. Color is displayed with 749/438 nm in red, 749/917 nm in green, and 438/749 nm in blue and is shown overlaid on a 749-nm image to retain morphologic information. In this color scheme, green areas indicate stronger absorption bands and red areas indicate steeper spectral slopes. Dark material, with

shallow absorption bands and spectral slopes, is observed at both craters and is shown in blue. (B) Enhanced color view of Cornelia crater with the same color scheme. The floors of both craters are distinct from their surroundings, suggesting a difference in physical properties or composition. (C) Spectra of the floors and local dark material at Marcia and Cornelia, shown with an average spectrum of Vesta for comparison.

and pit formation via dehydration is consistent with GRaND observations of lower H abundances immediately surrounding Marcia crater (20).

Rapid degassing of a volatile-bearing melt-breccia mixture is consistent with the observed morphologies of Vesta's pitted terrain. Slumping around some pits (Fig. 1) may be due to terrain being undercut as material is eroded during pit development. Pits that formed within the edges of crater wall slumps (fig. S1) may have continued to degas through the slumped material. Where wall slumps were thicker, they probably inhibited pit formation. Fresh craters with flat floor deposits, like those that host the pitted terrain, are rare on Vesta. Other large craters that expose dark material are either substantially more degraded or their formation on relatively steep slopes resulted in large, asymmetric slump deposits (5) that buried what would have been the crater floor. The sizes of pits on Vesta follow the Mars power-law trend of increasing pit size with host crater diameter (13). Pit size appears to be controlled by the thickness of the impact-heated deposit, expected to be similar on both bodies, rather than specific volatile content.

An alternative source of volatiles is impacting comets. As ice is not likely to be stable in sufficient volumes near Vesta's surface at equatorial latitudes (26), cometary volatiles would need to be delivered during the crater-forming event. However, the high impact velocities of comets typically result in vaporization and loss of the majority of the projectile, which suggests that little cometary material would be preserved within the crater floor and local ejecta. We also see evidence that pitted terrain may be tied to local surface properties rather than specific impactor composition. Though a small sample, pitted terrain is located only in regions not associated with the Rheasilvia impact basin, which dominates Vesta's southern hemisphere (27) and contains the lowest concentrations of hydrogen (20) and a dearth of dark material (6).

The formation mechanism for pitted terrain must explain the occurrence of these features on Mars and Vesta and their absence on the Moon, Mercury, and other asteroids for which images of comparable resolution exists. If delivery of hydrated material to the surface by impactors such as carbonaceous chondrites can result in the de-

velopment of pitted terrain, similar features should be observed on other volatile-poor airless bodies. Higher average impact velocities at Mercury and the Moon (28) may account for this difference, reducing the likelihood that impactors will be preserved without shock devolatilization. Pitted terrain may yet be discovered on other asteroids; among the handful already imaged at high resolution, the large size, complex geologic evolution, and chance collisional history of Vesta set it apart. Vesta's surface appears to be unique among airless bodies observed to date in the nature and degree of preservation of exogenic materials.

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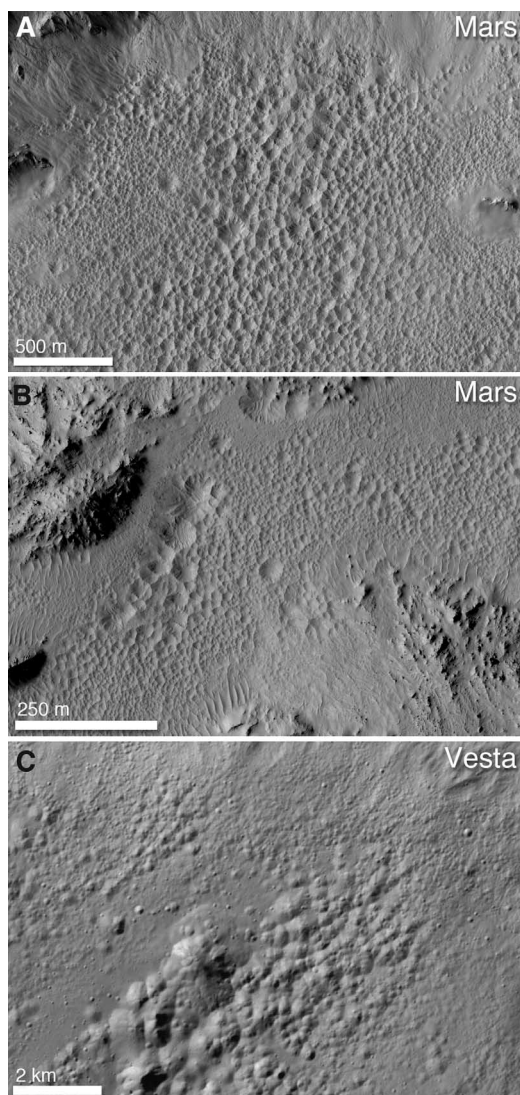
Acknowledgments: We thank the Dawn Science, Instrument, and Operations Teams and the Dawn at Vesta Participating Scientist program for support. A portion of this work was performed at the Jet Propulsion Laboratory, California Institute of Technology, under contract with NASA, and portions were supported by the Italian Space Agency. Dawn data are archived with the NASA Planetary Data System.

Supplementary Materials

www.sciencemag.org/cgi/content/full/science.1225374/DC1
Figs. S1 to S5
References (29–33)

30 May 2012; accepted 28 August 2012
Published online 20 September 2012;
10.1126/science.1225374

Fig. 4. Comparison between pitted terrain on Mars and Vesta. The morphologies of pits are similar, with irregular shapes and near-polygonal margins where pits share walls. (A) Floor of the 28-km crater Tooting, Mars [23.2°N, 207.8°E; High Resolution Imaging Science Experiment (HiRISE) image 1538_2035]. (B) The 10-km Zunil crater, Mars (7.7°N, 166.2°E; HiRISE image 1764_1880). (C) Marcia crater, Vesta. Whereas the examples shown here are at different scales, pits on Vesta fall on the martian trend for pit size versus crater diameter (13).



Sombrero Uplift Above the Altiplano-Puna Magma Body: Evidence of a Ballooning Mid-Crustal Diapir

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The Altiplano-Puna ultralow-velocity zone in the central Andes, South America, is the largest active magma body in Earth's continental crust. Space geodetic observations reported an uplift in the Altiplano-Puna proper at a rate of ~ 10 mm/year; however, the nature of the inferred inflation source has been uncertain. We present data showing that the uplift has persisted at a nearly constant rate over the past two decades, and is surrounded by a broad zone of subsidence. We show that the ongoing uplift and peripheral subsidence may result from a large mid-crustal diapir fed by partial melt from the Altiplano-Puna Magma Body.

There is a long-standing debate on the mechanisms of transport of silicic melts from the source region to the final emplacement levels in the upper crust. Once-prevailing views of slow transport of silicic melts in crustal diapirs (1) have been challenged by considerations of thermal viability of diapirs and suggestions that most of granitic melts are transported to the upper crust in dikes (2, 3). Subsequent theoretical studies have shown that granitic dikes may have difficulty leaving the source region (4) and that diapirs may not in fact be in danger of freezing if one relaxes simplifying assumptions about the rheology of the ductile lower crust—for example, by explicitly considering temperature-dependent power-law creep (5).

Magma transport through the crust may be accompanied by deformation of Earth's surface. Geodetic observations in neovolcanic areas commonly reveal episodes of uplift due to inflation of magma bodies in Earth's upper crust (6–11), possibly indicating injection of dikes from deeper sources. No observations of surface deformation due to magmatic diapirs have been reported so far. Expected features of diapir-related deformation include nearly constant surface velocities, large spatial wavelengths (on the order of tens of kilometers or more), axisymmetric patterns, and association with robust magmatic activity. Here, we investigated a long-term, long-wavelength crustal uplift in the Altiplano-Puna province in South America, a site of intense silicic volcanism over the past 10 million years. The Altiplano-Puna volcanic province belongs to an active volcanic arc in the central Andes, extending through Peru, southwestern Bolivia, Chile, and northwestern Argentina (12, 13). The province hosts a number of large calderas formed as a result of catastrophic eruptions. Seismic observations revealed that much of the volcanic province is underlain by a massive ultralow-velocity zone (ULVZ) at a depth of 17 to

19 km (14, 15). The Altiplano-Puna Ultralow-Velocity Zone (APULVZ) has thickness on the order of 1 km and lateral extent on the order of 100 km, and is believed to be the largest known active magma body in Earth's continental crust (15).

Space geodetic surveys using interferometric synthetic aperture radar (InSAR) identified surface uplift within the APULVZ proper (16). This uplift was attributed to the Uturuncu volcano, although that volcano has remained dormant over the past 2.7×10^5 years (16, 17). The surface uplift field with a spatial wavelength of tens of kilometers requires either a deep (depth > 15 km) or an areally extensive (characteristic diameter > 20 km) source of inflation (16). Because InSAR data from only one look direction are not sufficient to constrain the depth and geometry of pressurized magma bodies (10, 18), understand-

ing the nature of uplift in the Altiplano-Puna province requires measurements of both the vertical and horizontal components of deformation.

To reduce uncertainties in the depth estimates of the inferred inflation source, in 2006 we asked the European Space Agency to task the ERS-2 and Envisat satellites to acquire data from both the ascending and descending orbits over the APULVZ. Several tens of acquisitions were made, increasing the total time span of observations from 1992 until 2010. Average line-of-sight (LOS) velocities from several satellite tracks covering APULVZ (Fig. 1) indicate that the central uplift near the Uturuncu volcano is surrounded by a broader region of subsidence at a rate that is much smaller than the peak uplift rate (19). The peripheral subsidence was not detected in previous studies, presumably because of a shorter time span of observations and a lower signal-to-noise ratio. We refer to the observed deformation pattern (Fig. 1) as the “sombrero uplift.” Analysis of time dependence of surface unrest indicates that the latter has persisted over the past two decades (Fig. 2 and fig. S2). The monotonic uplift in the center of the APULVZ contrasts with episodic inflation typical of shallow magma bodies in the upper crust (7, 11, 20) but is similar to deformation associated with another large mid-crustal magma body in the Rio Grande Rift in New Mexico (21, 22).

We used the average LOS velocities from different radar look directions to constrain the depth of the inflation source (fig. S2). Joint inversions of average LOS velocities are subject to several uncertainties because of the relative nature of

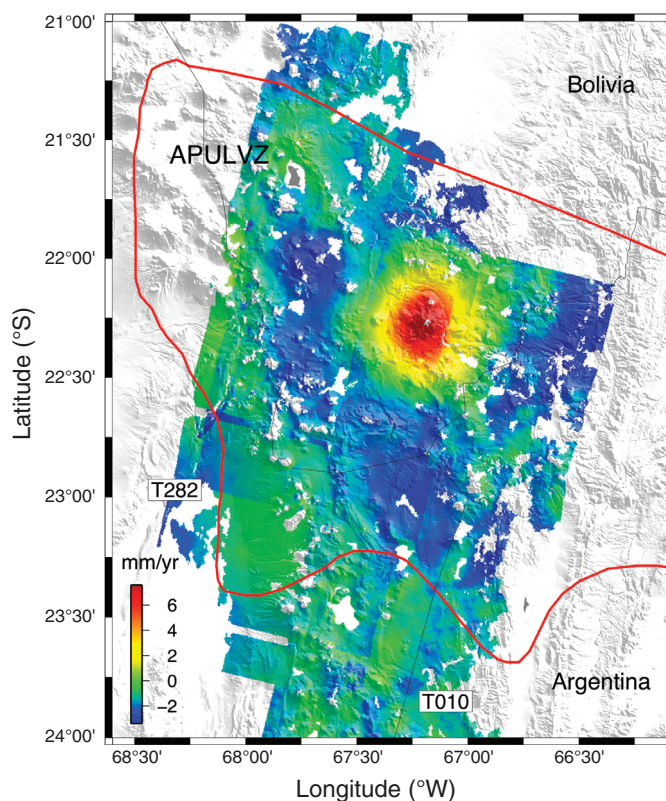


Fig. 1. Mosaic of LOS velocities obtained from stacking of ERS-1/2 and Envisat data from the descending tracks 282 and 10. Motion toward the satellite is taken to be positive. The LOS velocity is constrained to have a zero mean value in the far field, away from the imaged deformation anomaly. Note a ring of subsidence surrounding the central uplift. Red line denotes the extent of the seismically imaged ULVZ in the middle crust (14, 15).

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InSAR measurements, different time spans of acquisitions from different satellite tracks, and possible temporal variations in the uplift rate. In addition, in cases of superposition of the central uplift and peripheral subsidence (Fig. 1), inversions that consider a single source of inflation result in underestimation of the source depth.

To avoid biases due to the source complexity, we use positions of the LOS velocity maxima that are independent of the estimated uplift rates. The data from the descending (fig. S2, A and C) and ascending (fig. S2B) satellite orbits show that peaks in the LOS velocity anomalies are separated by as much as 8 ± 2 km. This separation stems from the contribution of horizontal displacements to changes in the radar range (10). Forward modeling indicates that the separation increases with the source depth for a given source geometry; for oblate (sill-like) sources, it also increases with the source diameter for a given source depth. Models assuming an oblate source geometry and elastic deformation of the host rocks (23) fail to produce the observed separation between peaks in the LOS velocities (fig. S2); the maximum predicted separation is 3 to 5 km for sills shallower than 12 km. This result implies that (i) the inflation source may be spheroidal or prolate in shape, (ii) the source is located below the brittle-ductile transition, and (iii) the assumption of purely elastic deformation is invalid.

We performed numerical experiments to evaluate the effect of stress relaxation in the middle crust on surface velocities (19). We found that the main effect of viscous deformation is to reduce

the contribution of the horizontal component of the velocity field with respect to the vertical component (fig. S4). A smaller ratio of horizontal to vertical displacements translates into a smaller separation between the maxima in the LOS displacements from the descending and ascending orbits, which lends further support to the inference of a prolate source geometry. We note that shallow (depth <15 km) isometric or prolate sources in elastic half-space can be ruled out, as they are not able to generate an uplift anomaly of sufficiently large wavelength. An upper bound on the source depth is likely provided by the seismically imaged ULVZ. This is because the massive partially molten APULVZ (14, 15) is likely to absorb strain from hypothetical underlying sources, thereby precluding a localized deformation at Earth's surface. The same argument can be invoked against a deep deflation source as a possible cause of peripheral subsidence (Fig. 1).

Although it is possible to fit the InSAR data by a simple elastic half-space model consisting of two point sources of volume change (Mogi sources; fig. S3), such a model may be deemed unreasonable on the basis of the following considerations. First, the inflating and deflating Mogi sources would have to reside at depths of at least 25 and 80 km, respectively, to explain the observed wavelength of surface deformation (Fig. 1 and fig. S3). These source depths are greater than those of the brittle-ductile transition and the APULVZ (14, 15), so that the assumption of elastic deformation is inapplicable, as discussed above. Second, the inferred rate of deflation of

the hypothesized deep source exceeds the inflation rate of the shallower source by a factor of 4 or more, raising an issue of mass balance. Third, a long-term surface uplift (Fig. 2) would imply either a permanent magma conduit with a constant flow rate connecting the upper mantle source to the mid-crustal magma body, or a quasi-steady supply of melt to the mid-crustal magma body via frequent magma-driven fractures. Mechanical and thermal considerations suggest that such scenarios are unlikely (19). Alternatively, the persistent nature of surface deformation may be attributed to slow viscous deformation induced by a large magma body in the middle crust (22).

We explored the possibility that the observed sombrero uplift is associated with the Altiplano-Puna Magma Body itself. As an initial test of this hypothesis, we ran a series of inverse models using a combination of an inflating source and a grid of deflating Mogi sources at the seismically inferred depth of the APULVZ. The grid was designed to match the extent of the observed surface subsidence (Fig. 1). An inflating source was represented by a prolate spheroid (24, 25). The depth of the inflating source deduced from these inversions was not resolvably different from the assumed depth of the distributed deflating source representing the APULVZ. Furthermore, the rate of volume change was found to be approximately equal for the inflating and deflating sources. Although this simple kinematic model is able to fit the data, it does not explain the mechanism of volume changes at depth. Note that a sill-like body with interconnected melt cannot maintain substantial lateral gradients in the magma pressure (e.g., an overpressure in the center of the body and an underpressure elsewhere).

Instead, we propose that the sombrero uplift (Fig. 1) results from ballooning of a large diapir fed by hot low-viscosity material from the APULVZ. One possibility is that partial melting in the APULVZ produces magma of lower density relative to the host rocks, which may result in the Rayleigh-Taylor instability in the roof of the magma body and formation of a buoyant diapir. As such a diapir increases in size, it may be fed by lateral migration of partial melt within the APULVZ. According to this model, inflation of the buoyant diapir causes central uplift, and withdrawal of material from the parental APULVZ is responsible for the peripheral subsidence. Note that the optimal location for the development of a diapir is in the middle of the APULVZ, consistent with seismologic and geodetic observations (Fig. 1). The inferred depth of the magma source is also consistent with available petrological constraints (17).

To test this hypothesis, we performed time-dependent 3D numerical simulations of deformation due to a buoyant diapir in the center of the APULVZ. Simulations were carried out using a finite element code, Abaqus (26). The model domain is a cylinder with a radius of 300 km and thickness of 200 km. The model includes an elastic crust with thickness of 12 km and a viscoelastic substrate. The viscoelastic substrate obeys the

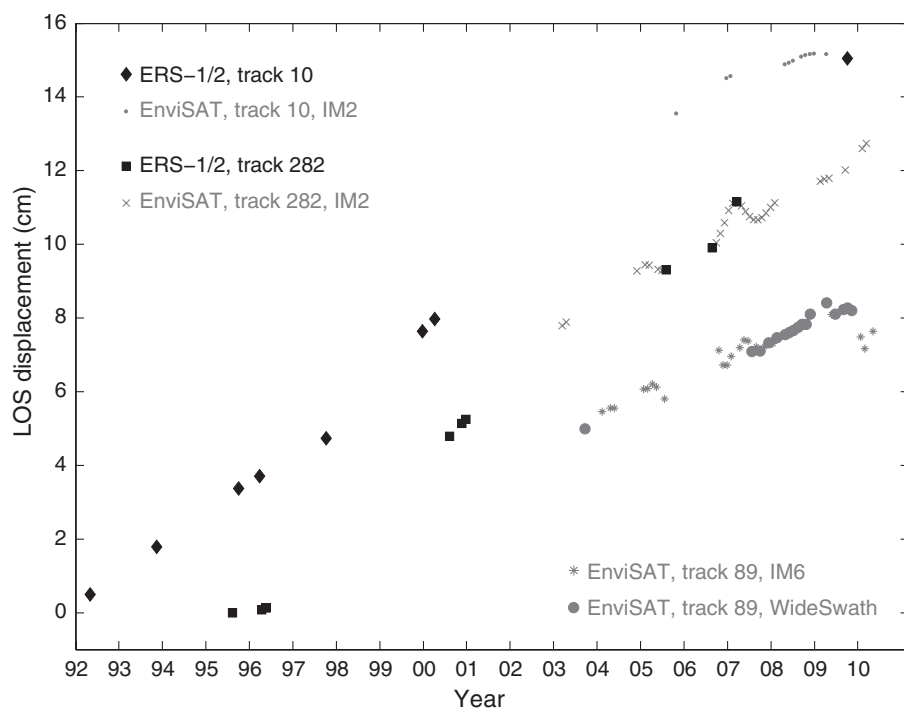


Fig. 2. Time series of LOS displacements calculated using data from the ERS (black symbols) and EnviSAT (gray symbols) acquisitions from tracks 10 (diamonds and dots), 282 (squares and crosses), and 89 (circles and asterisks). Origin of the time series is arbitrary. Locations of the reference sites are denoted by the respective symbols in fig. S2. Small-scale subannual undulations seen in LOS displacements are most likely of atmospheric origin.

temperature-dependent power-law rheology with laboratory-derived material parameters [see (19) for details of numerical implementation and material parameters]. The source region is approximated by a tabular body having the areal extent of the APULVZ (Fig. 1) and thickness of 1.5 km. The upper boundary of the source region is located at a depth of 19 km below the free surface. The buoyant region is approximated by a semi-ellipsoid of rotation with vertical semi-axis of 6.5 km and horizontal semi-axis of 5 km, extending 5 km above the upper boundary of the APULVZ (fig. S6). Both the diapir and the tabular source region representing the APULVZ are prescribed a linear Maxwell viscoelastic rheology. The material within the diapir has a lower density relative to the host rocks. The material within the source region is assumed to be neutrally buoyant, so that it is passively entrained by the ascending diapir. Depending on the value of melt fraction in the APULVZ, lateral transport of melt toward the central upwelling may occur as either channel or porous flow. We approximate both processes by allowing bulk viscous deformation inside the diapir and the tabular source region to ensure conservation of mass. Figure 3 shows predictions of the best-fitting “ballooning diapir” model. As can be seen in Fig. 3, the wavelength, pattern, and amplitude of predicted surface velocities are in good agreement with InSAR observations. The peak uplift velocity predicted by our best-fit model is on the order of 10 mm/year after ~20 years of deformation, and gradually decreases with time. The predicted separation between

peak LOS velocities corresponding to the ascending and descending satellite orbits is 6 km, in overall agreement with InSAR observations (fig. S2).

Our observations and modeling results therefore suggest that the ongoing sombrero uplift in the Altiplano-Puna province (Fig. 1) manifests as the formation of a large diapir in the roof of the Altiplano-Puna Magma Body. It is instructive to compare deformation due to the APULVZ to that due to the Socorro Magma Body (SMB) in central New Mexico, arguably the second largest magma body in Earth's continental crust (27). The two magma bodies occur in very different tectonic settings but nonetheless share a number of remarkable similarities. Both magma bodies are located in the middle crust just above a depth of 20 km. Both bodies are associated with seismic activity in the upper crust, and the long-term uplift rate is on the order of millimeters per year (17, 22, 23, 28). In both cases, the modeled source of inflation is smaller than the seismically imaged magma body (22). There is indication of subsidence around the central uplift due to the SMB (10, 21, 22), although the rates of both uplift and subsidence due to the SMB are smaller than those due to the Altiplano-Puna Magma Body and therefore subject to greater uncertainties (Fig. 3). These similarities, along with modeling results presented in this study, suggest that the uplift and peripheral subsidence due to the SMB might also manifest as the formation of a magmatic diapir, rather than viscous response to inflation of a large sill-like magma body, as suggested previously (10, 22). Models predict

that in both cases the surface uplift should gradually slow down and increase in wavelength, provided there is no supply of fresh melt from a deeper (e.g., mantle) source. However, the details of surface deformation (e.g., the ratio of maximum horizontal to maximum vertical displacements; fig. S4) may be sufficiently different for the sill and diapir models. The competing hypotheses therefore can be tested with further observations at the Altiplano-Puna and Socorro sites, as well as other areas of long-term uplift due to mid-crustal magma bodies. In the absence of direct observations of magma transport at depth, space geodetic surveys in neovolcanic areas can provide critical constraints on the occurrence, time scale, and dynamics of magmatic diapirism.

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Acknowledgments: Supported by NSF grant EAR-0944336. SAR data are available from the European Space Agency (eopi.esa.int).

Supplementary Materials

www.sciencemag.org/cgi/content/full/338/6104/250/DC1
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Supplementary Text
Figs. S1 to S6
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20 June 2012; accepted 27 August 2012
10.1126/science.1226358

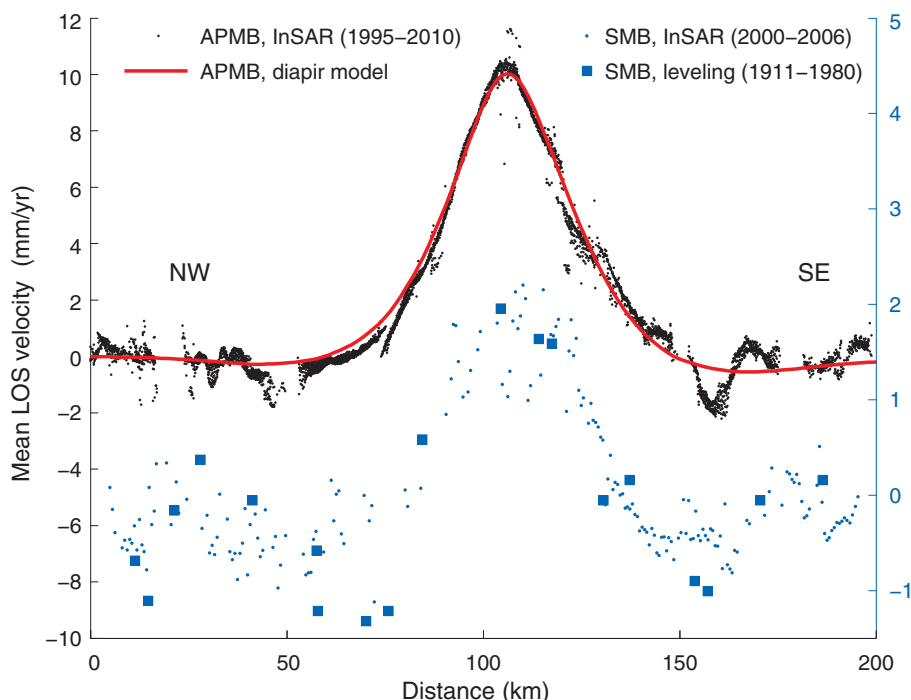


Fig. 3. Observed (black dots) and predicted (solid red line) LOS velocities along the northwest-to-southeast profile crossing the center of the uplift (Fig. 1). The model predictions correspond to a buoyant diapir at the top of the APULVZ, with density contrast of 400 kg m^{-3} . Shown at the bottom are surface velocities due to the Socorro Magma Body (New Mexico) from InSAR and leveling measurements (blue symbols, right axis).

Adhesion Functions in Cell Sorting by Mechanically Coupling the Cortices of Adhering Cells

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Differential cell adhesion and cortex tension are thought to drive cell sorting by controlling cell-cell contact formation. Here, we show that cell adhesion and cortex tension have different mechanical functions in controlling progenitor cell-cell contact formation and sorting during zebrafish gastrulation. Cortex tension controls cell-cell contact expansion by modulating interfacial tension at the contact. By contrast, adhesion has little direct function in contact expansion, but instead is needed to mechanically couple the cortices of adhering cells at their contacts, allowing cortex tension to control contact expansion. The coupling function of adhesion is mediated by E-cadherin and limited by the mechanical anchoring of E-cadherin to the cortex. Thus, cell adhesion provides the mechanical scaffold for cell cortex tension to drive cell sorting during gastrulation.

Cell adhesion and cortex tension are commonly assumed to function in cell sorting by controlling cell-cell contact formation (1–5), with adhesion increasing the contact size and cortex tension decreasing it (3–7). To clarify how cell adhesion and cortex tension function in

progenitor cell-cell contact formation and sorting during zebrafish gastrulation, we first developed a mechanical description of two progenitor cells in contact, on the basis of previous models of cell-cell adhesion and sorting (4, 5). The cells are described as fluid objects with a viscoelastic

cortex under tension and adhesive bonds maintaining the cell-cell contact. The size of the cell-cell contact is determined by the balance of forces at the contact boundary:

$$\cos(\theta) = \frac{\gamma_i}{2\gamma_{cm}} = \frac{2\gamma_{cc} - \omega}{2\gamma_{cm}} \quad (1)$$

where θ is the contact angle of the two adhering cells (Fig. 1A) (8, 9). The tension γ_i at the cell-cell interface has a positive contribution arising from the cortex tension γ_{cc} of the two cells at the contact and a negative contribution arising from adhesion (adhesion tension) of magnitude ω . Outside of the contact, the tension at the cell-medium interface is equal to the cortex tension γ_{cm} at this interface.

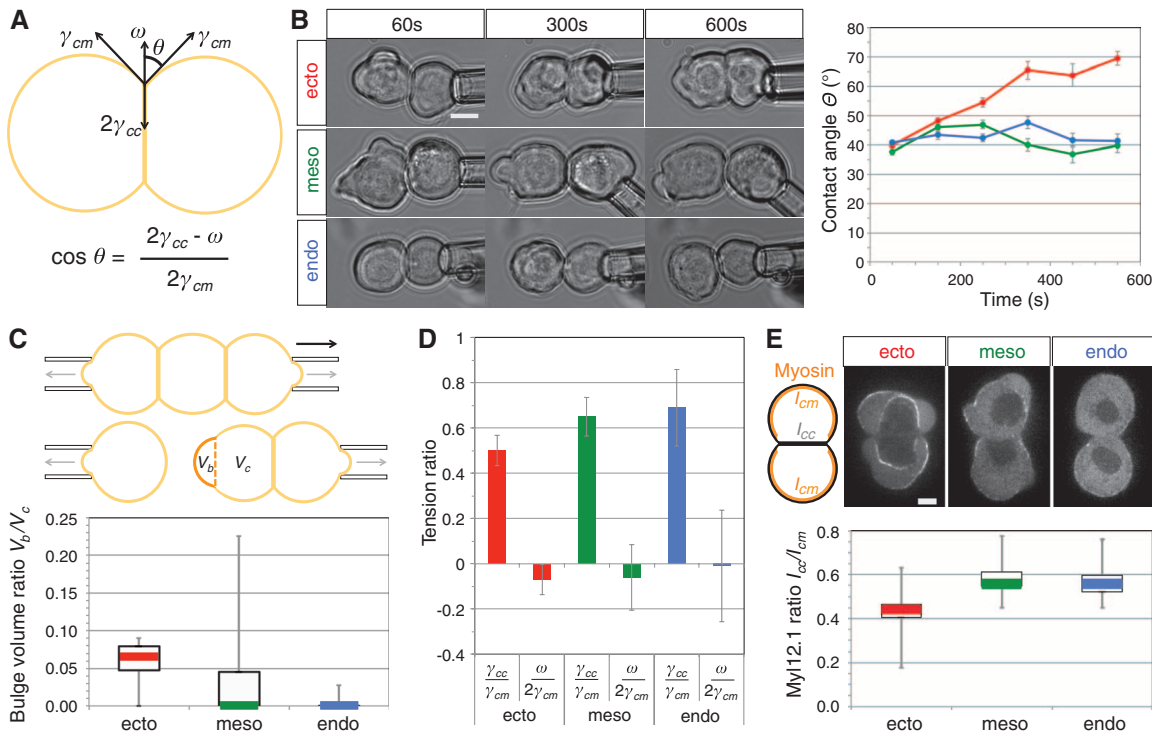
To characterize the mechanical parameters that control progenitor cell-cell contact formation, we first determined the ratio of the

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Fig. 1. Surface tensions and contact shape in progenitor cell doublets.

(A) Surface tensions controlling cell doublet shape at steady state. The contact angle θ results from the balance between the adhesion tension ω and the cortex tensions at the cell-medium γ_{cm} and cell-cell interfaces γ_{cc} (9). $\cos \theta = \frac{2\gamma_{cc} - \omega}{2\gamma_{cm}}$. **(B)** Homotypic ectoderm (ecto), mesoderm (meso), and endoderm (endo) doublets during contact formation (movies S1 to S3). Scale bar, 10 μ m. Measured contact angles θ are plotted over time as mean $\pm$ SEM binned over 100 s for ecto (red, $n = 39$), meso (green, $n = 20$), and endo (blue, $n = 26$) doublets (table S1). **(C)** Sketch of homotypic triplets before and after separation (9). Bulge volume V_b is measured at the former cell-cell contact after separation and normalized to the cell body volume V_c for ecto ($n = 11$), meso ($n = 13$), and endo ($n = 5$) triplets (movies S4 to S6 and table S2). **(D)** Tension ratios γ_{cc}/γ_{cm} and $\omega/2\gamma_{cm}$ computed from homotypic triplet and doublet shapes plotted for ecto ($n = 11$), meso ($n = 13$), and endo ($n = 5$). Mean $\pm$ SEM (table S3) (9). **(E)** Sketch of



myosin [Myl12.1-eGFP (enhanced green fluorescent protein)] localization in homotypic doublets of pTol2- β -actin::myl12.1-eGFP transgenic zebrafish. Measured mean fluorescence intensity at the cell-cell interface (I_{cc}) is normalized to the mean intensities at the cell-medium interfaces (I_{cm}) of both cells for ecto ($n = 25$), meso ($n = 33$), and endo ($n = 17$) doublets. Scale bar, 5 μ m.

Fig. 2. Contact strength and structure in progenitor cell doublets. **(A)** Separation force F_s of ectoderm (red; $n = 104/41/18$), mesoderm (green; $n = 30/16/11$), and endoderm (blue; $n = 44/23/16$) homotypic doublets is plotted as mean $\pm$ SEM at 1-, 5-, and 10-min contact times (movie S7 and table S4) (9). **(B)** Ratio of separation force F_s to contact radius R_c of ecto ($n = 37$), meso ($n = 15$), and endo ($n = 25$) homotypic doublets after 5-min contact time (table S5). **(C)** Sketch of a cell doublet showing cadherin (purple), actin (cyan), and myosin (orange). Optical sections through cell-cell contacts of homotypic doublets stained with antibodies against Cdh1, Ctnnb1, and Ctnna; phalloidin for F-actin; or expressing Myl12.1-mCherry (fig. S3). Scale bar, 5 μm .

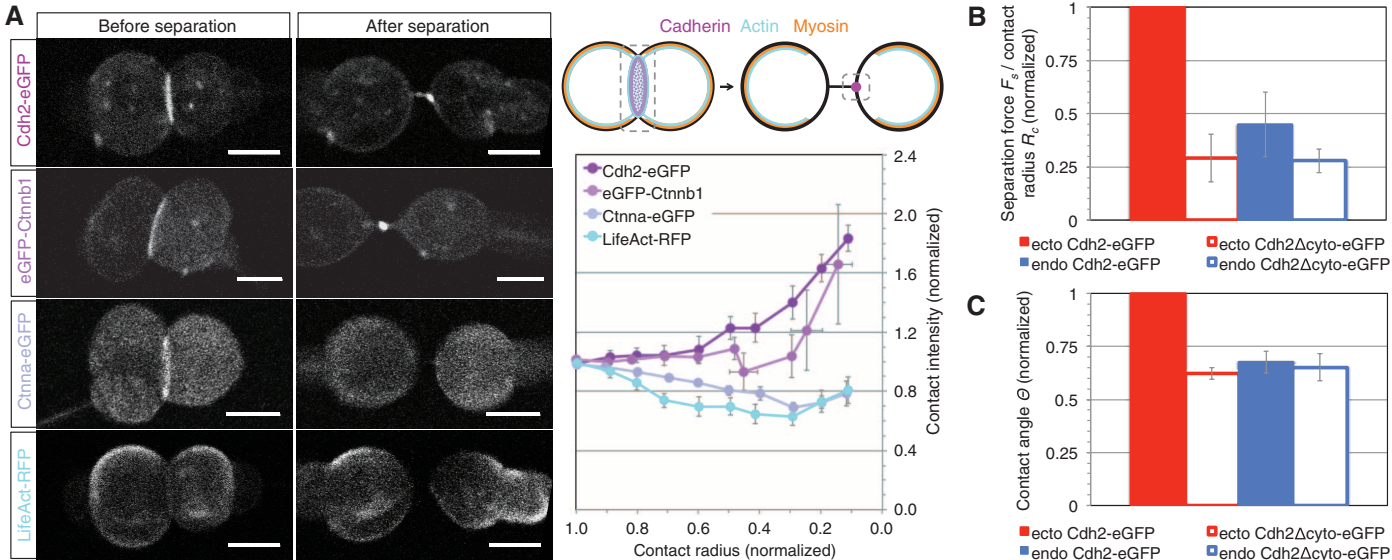
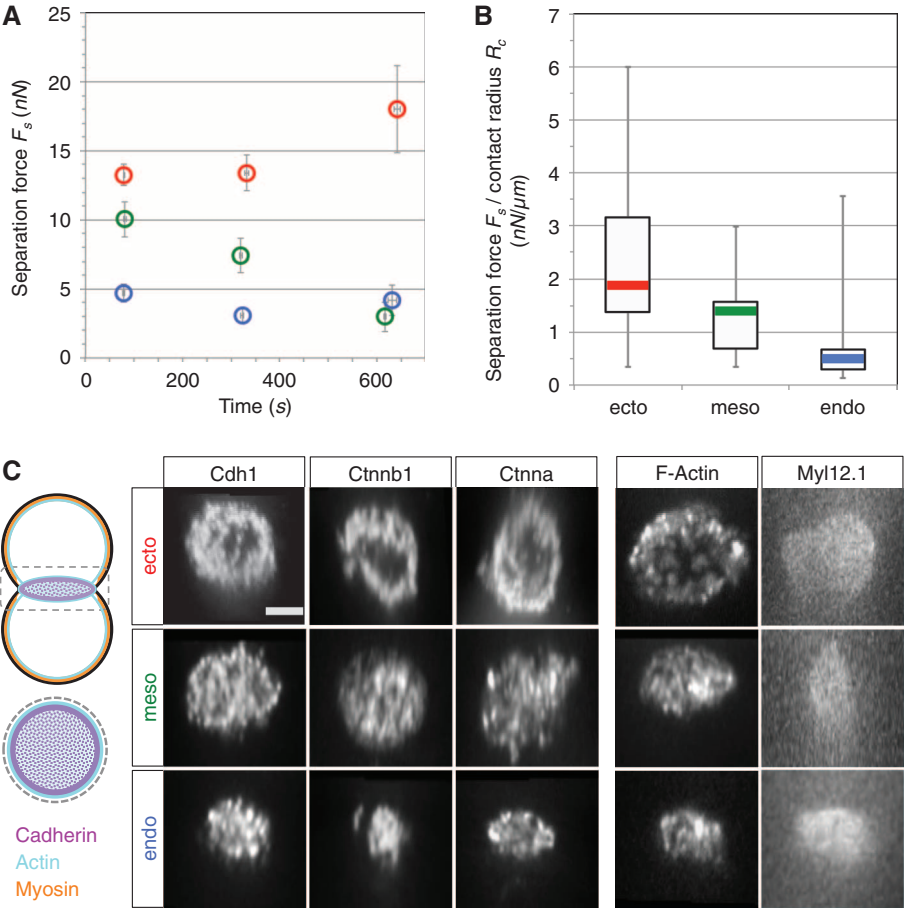


Fig. 3. Cytoskeletal anchoring of Cdh2 in progenitor cell-cell contact formation. **(A)** Ectoderm homotypic doublets expressing Cdh2-eGFP plus LifeAct-RFP (red fluorescent protein) ($n = 15$), eGFP-Ctnnb1 ($n = 20$), or Ctnna-eGFP ($n = 30$) at 5 min contact time before and after separation (movies S8 to S11). Fluorescence intensity at the contact and contact size measured during the separation process (purple and/or cyan on diagram) are plotted as mean $\pm$ SEM relative to the value before the separation

(table S6). Scale bars, 10 μm . **(B and C)** Ratio of separation force to contact radius F_s/R_c (B) or contact angle θ (C) measurements of ectoderm (red) or endoderm (blue) homotypic doublets expressing Cdh2-eGFP ($n = 17/20$) or Cdh2 Δ cyto-eGFP ($n = 20/14$) at 5-min contact time. Values are plotted as mean $\pm$ SEM and normalized to Cdh2 expression level at the measured contact and at the contact of ecto doublets (movies S12 to S15 and tables S7 and S8).

interfacial tensions at the cell-cell and cell-medium interfaces by measuring the contact angle θ of freely adhering cell doublets *ex vivo* and using Eq. 1. Homotypic ectoderm doublets showed a larger contact angle θ and consequently a lower ratio of cell-cell to cell-medium interfacial tensions $\gamma_i/2\gamma_{cm}$ than mesoderm and endoderm doublets (Fig. 1B).

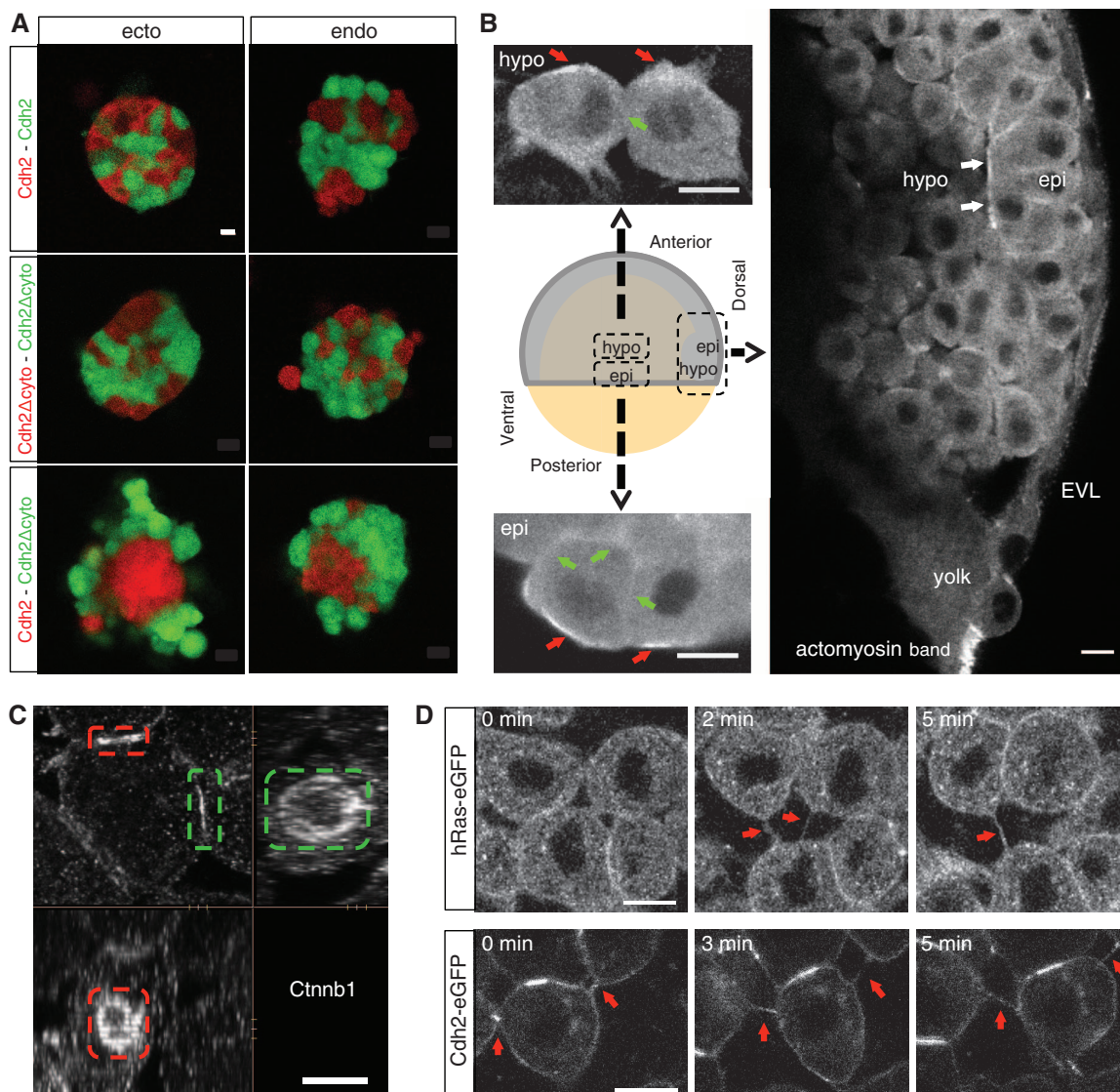
To derive the relative contribution of the cortex tension γ_{cc} and the adhesion tension ω to the ratio of cell-cell to cell-medium interfacial tensions $(2\gamma_{cc} - \omega)/2\gamma_{cm}$, we measured the ratio of cortex tensions at the cell-cell to cell-medium interfaces γ_{cc}/γ_{cm} . To this end, we probed cortex tension at the cell-cell interface by separating contacting progenitor cells *ex vivo*, using a dual pipette aspiration assay (DPA) and analyzing resulting shape changes directly after separation (Fig. 1C). We observed a rapid increase in cur-

vature in the region of the dissolved cell-cell contact, indicative of reduced cortex tension γ_{cc} at this location compared to cortex tension γ_{cm} at the cell-medium interface. To calculate the ratio of cortex tensions γ_{cc}/γ_{cm} from the curvature change at the dissolved cell-cell contact in the different progenitor cell types, we modeled the cell-cell and cell-medium interfaces as elastic shells under tension, consistent with the short time scales of our experiment (9). Using this model, we found γ_{cc}/γ_{cm} to be lower in contacting ectoderm cells compared to mesoderm and endoderm cells (Fig. 1D). Consistent with this, we observed that in progenitor cell doublets, non-muscle myosin-2 (Myl12.1) was reduced at cell-cell compared to the cell-medium interfaces, and that this reduction was more pronounced in ectoderm compared to mesoderm and endoderm doublets (Fig. 1E).

Having determined the ratio of tensions at the cell-cell and cell-medium interfaces, we computed the adhesion tension ω using Eq. 1. We found that for all three progenitor cell types, the magnitude of the adhesion tension ω was considerably smaller than the cortex tension γ_{cc} at the cell-cell interface (Fig. 1D). This indicates that the cell-cell interfacial tension γ_i is dominated by the cortex tension γ_{cc} at this interface. It further suggests that the cell-cell contact angle θ , and thus the contact size, is predominantly controlled by the ratio of cortex tensions γ_{cc}/γ_{cm} between these interfaces and that adhesion tension ω , contrary to previous suggestions (8), has only little function in contact expansion.

Although these findings argue against a critical function of adhesion tension in cell-cell contact expansion, formation of adhesive bonds is still essential to mechanically couple the contractile

Fig. 4. Progenitor cell sorting in vitro and cell-cell contacts structure in vivo. (A) Sorting of red- or green-labeled Cdh2-eGFP- or Cdh2 Δ cyto-eGFP-expressing progenitors in ectoderm or endoderm cell aggregates (movies S16 to S19 and fig. S7). Scale bar, 10 μ m. (B) Myl12.1-eGFP localization within the shield region of pTol2- β -actin::myl12.1-eGFP transgenic zebrafish at 6 hours postfertilization (hpf) (right to the sketch); arrows demarcate the epiblast-hypoblast boundary (epi-hypo, movie S20). Exemplary cells are shown within the lateral mesendoderm (top) and at the germ ring margin (bottom). Red arrows point to Myl12.1 accumulation at the cell-interstitial space interface, and green arrows to Myl12.1-depleted zones at the cell-cell interfaces (fig. S8). Scale bars, 10 μ m. (C) Ring-like accumulation of Ctnnb1 at the contact margin between epiblast cells at the animal pole revealed by antibody staining (movie S21). Boxes highlight the contact on the imaging planes and orthogonal views. Scale bar, 10 μ m. (D) Membrane tethers (arrows) formed between separating cells within the lateral mesendoderm in 7-hpf embryos expressing hRas-eGFP (top, movie S22) or within the animal pole of shield-stage embryo expressing Cdh2-eGFP (bottom, movie S23). Scale bars, 10 μ m.



cortices of the adhering cells at the contact and to support stresses normal to the adhesion zone arising from cortical tension, intracellular pressure, or external forces (10, 11). Notably, this mechanical coupling function of adhesion is distinct from the role adhesion plays in providing the adhesion tension ω , where it acts in the direction tangential to the contact zone, thereby expanding it. To characterize the function of mechanical coupling due to adhesion in progenitor cell-cell contact formation and sorting, we asked how its strength is controlled in the different progenitor cell types, and whether interfering with its strength affects progenitor cell-cell contact formation and cell sorting.

To determine the strength of mechanical coupling due to adhesion, we used the DPA assay to mechanically separate homotypic progenitor cell doublets ex vivo and measure the corresponding separation force (12). Considering that cell-cell separation was achieved rapidly (<1 s), our experiments can be described as the separation of two elastic solids, where the separation force F_s depends on different factors, including the number and dissociation rate of adhesion bonds (9, 13). We found that ectoderm doublets exhibit higher F_s at contact times varying from 1 to 10 min than mesoderm and endoderm doublets (Fig. 2A). This difference in F_s between the progenitor cell types is not just a consequence of differences in cell-cell contact size, because normalizing F_s by the contact radius (F_s/R_c) (9) still yielded higher values for ectoderm compared to mesoderm and endoderm doublets (Fig. 2B and fig. S1). Differences in adhesion molecule density at the contact are also unlikely to account for the different F_s , as the expression levels of E-cadherin (Cdh1) and associated proteins, previously shown to mediate adhesion in all three progenitor cell types (1), did not correlate with the different F_s in these cells (fig. S2).

We next asked whether differences in the mechanical resistance of adhesion bonds to pulling forces, previously implicated in controlling F_s (12), are responsible for the differences in F_s between the progenitor cell types. To this end, we first determined whether the binding strength of cadherins across the cell-cell contact or to the cortical cytoskeleton limits their mechanical resistance by analyzing the segregation of adhesion complex components during cell-cell separation. Before separation, Cdh1, β -Catenin (Ctnnb1), α -Catenin (Ctnna), and actin, but not Myl12.1, were found to accumulate in a dense ring-like structure at the margin of the cell-cell contact in ectoderm and, to a lesser extent, mesoderm and endoderm doublets (Fig. 2C and figs. S3 and S4). This suggests that higher F_s values coincide with adhesion molecules accumulating at the contact edge. Upon separation by means of the DPA, progenitor cells remained connected via long plasma membrane tethers, and N-cadherin (Cdh2; see below for using Cdh2 as a proxy for Cdh1) together with Ctnnb1, which directly binds to Cdh2 (10),

accumulated in these tethers (Fig. 3A). By contrast, Ctnna, which directly or indirectly couples Ctnnb1 to the actin cytoskeleton (14, 15), did not colocalize with Cdh2 and Ctnnb1 in the tethers, but instead disassembled from the dissolving cell-cell contact together with actin (Fig. 3A). These findings indicate that cadherins dissociate from the cytoskeleton during progenitor cell separation, suggesting that cytoskeletal anchoring of cadherins limits the mechanical resistance of adhesion bonds to pulling forces.

To explore whether modifying cytoskeletal anchoring of cadherins, and thus the mechanical resistance of adhesion bonds to pulling forces, affects F_s and cell-cell contact formation, we expressed either a full-length version of Cdh2 or a truncated version of Cdh2 that cannot bind to the cortical cytoskeleton (Cdh2 Δ cyto) (12) in progenitor cells deprived of endogenous Cdh1 expression (cdh1 morphant cells). Expressing similar levels of Cdh2 in homotypic ectoderm and endoderm cell doublets changed neither their endogenous relative difference in F_s normalized by the contact radius F_s/R_c nor their contact angle θ . However, expressing Cdh2 Δ cyto strongly reduced F_s/R_c and θ to similar low levels in both cell types (Fig. 3, B and C, and figs. S5 and S6). This suggests that proper cytoskeletal anchoring of cadherins is an essential factor determining the difference in separation force and cell-cell contact size between the progenitor cell types.

We next asked how far the observed effect of cadherin cytoskeletal anchoring on F_s/R_c and θ influences progenitor cell sorting. Consistent with a critical function of cell-cell contact formation in cell sorting, replacing endogenous Cdh1 with either full-length or truncated Cdh2 in homotypic ectoderm or endoderm progenitor cell aggregates led to cells expressing Cdh2 being sorted into the middle, surrounded by cells expressing Cdh2 Δ cyto (Fig. 4A). These observations suggest that proper cytoskeletal anchoring of cadherins is essential for progenitor cell sorting.

To address how our observations of cell doublets ex vivo relate to the situation within the gastrulating embryo, we asked whether progenitor cells segregate in vivo by using the same cell-cell contact formation strategies as their counterparts ex vivo. Analysis of the cell cortex in germ layer progenitor cells in vivo showed that similar to what we observed ex vivo, cortical Myl12.1 was preferentially located at cell-interstitial space/extracellular matrix interfaces (Fig. 4B). This suggests that cortex tension is reduced at cell-cell interfaces both ex vivo and in vivo. Moreover, analysis of progenitor cell-cell contacts in vivo revealed a ring-like localization of adhesion molecules reminiscent of the situation in cell doublets ex vivo (Fig. 4C). Finally, progenitor cells separating in vivo often formed membrane tethers at their dissolving contacts containing Cdh2 (Fig. 4D), similar to cell doublets ex vivo that were separated by means of

the DPA. These marked similarities between the situations ex vivo and in vivo suggest that progenitor cell sorting in vivo is driven by the same mechanisms of cell-cell contact formation as observed ex vivo.

The mechanical coupling function of cadherins at the cell-cell contact critically requires sufficient anchoring of cadherins to the cortical cytoskeleton. Considering that mechanical load on cadherin adhesion complexes has previously been suggested to modify its cytoskeletal anchoring strength (15, 16), it is intriguing to speculate that this anchoring strength in the different progenitor cell types is determined by the cortex tension of these cells pulling on the mechanosensitive adhesion complexes, thereby setting their anchoring strength.

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Acknowledgments: We thank M. Biro, J.-Y. Tinevez, and J. Rönsch for technical assistance, V. Barone for critical reading of earlier versions of this manuscript, and the facilities of the MPI-CBG and IST Austria for their continuous support. This work was supported by the Max-Planck-Society and grants from the Polish Ministry of Science and Higher Education to E.P. (454/N-MPG/2009/0), the Human Frontier Science Program to E.P. (RGY0067/2008), and the Fonds zur Förderung der wissenschaftlichen Forschung (FWF) to C.-P.H. (HE3231/6; I812-B12).

Supplementary Materials

www.sciencemag.org/cgi/content/full/science.1225399/DC1
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Movies S1 to S23
30 May 2012; accepted 1 August 2012
Published online 23 August 2012;
10.1126/science.1225399

Forces Driving Epithelial Spreading in Zebrafish Gastrulation

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Contractile actomyosin rings drive various fundamental morphogenetic processes ranging from cytokinesis to wound healing. Actomyosin rings are generally thought to function by circumferential contraction. Here, we show that the spreading of the enveloping cell layer (EVL) over the yolk cell during zebrafish gastrulation is driven by a contractile actomyosin ring. In contrast to previous suggestions, we find that this ring functions not only by circumferential contraction but also by a flow-friction mechanism. This generates a pulling force through resistance against retrograde actomyosin flow. EVL spreading proceeds normally in situations where circumferential contraction is unproductive, indicating that the flow-friction mechanism is sufficient. Thus, actomyosin rings can function in epithelial morphogenesis through a combination of cable-constriction and flow-friction mechanisms.

In zebrafish epiboly, the enveloping cell layer (EVL) surface epithelium formed at the animal pole of the gastrula (1) spreads over the entire yolk cell to completely engulf it at the end of gastrulation. EVL movements are independent of epiboly movements of the deep cells below (2), but require contact between the EVL and the yolk syncytial layer (YSL) to which the EVL is connected at its margin (3, 4). Contractile actomyosin rings drive many fundamental morphogenetic processes (5–9), and it has been proposed that in

zebrafish a contractile actomyosin ring within the YSL, a thin cytoplasmic layer at the surface of the yolk cell, drives EVL epiboly by pulling on the edge of the EVL (4, 10, 11). However, the force-generating mechanisms by which this ring drives EVL epiboly movements remain elusive.

To investigate the physical mechanisms driving EVL epiboly, we first examined the global distribution of actin and myosin-2 within the embryo (movies S1 and S2). Just after the onset of epiboly, when the EVL covers 40% of the spherical yolk cell (40% epiboly; 5 hours post-fertilization, hpf), we observed an accumulation of both actin and myosin-2 within the YSL in a band-like structure close to the margin of the EVL (Fig. 1A). This initially broad actomyosin band ($85.8 \pm 11.8 \mu\text{m}$; errors denote error of the mean at 95% confidence unless noted otherwise) decreases its width during EVL epiboly progression, eventually forming a tight circumfer-

ential ring-like structure ($32.2 \pm 3.5 \mu\text{m}$) at 70 to 80% epiboly (7.5 to 8.5 hpf; Fig. 1, A to C).

We then asked if the YSL actomyosin ring drives EVL epiboly movements. Previous studies on the function of the mitogen-activated protein kinase (MAPK) pathway components *traf2/nika* (4) and *mapkapk2* (11) suggested that actomyosin contractility within the YSL affects EVL epiboly movements. To directly test whether YSL actomyosin contractility drives EVL epiboly, we locally disrupted the YSL actomyosin ring close to the EVL margin in 60% epiboly-stage embryos (6.5 hpf) with an ultraviolet (UV) laser cutter (12). This caused considerable delays in epiboly movements of the EVL margin directly adjacent to the ablation site (Fig. 1, D to F, and movie S3), suggesting that the YSL actomyosin ring is required for EVL epiboly movements and that its functional requirement is locally restricted.

We next sought to understand how YSL actomyosin ring contractility drives EVL epiboly movements. One possibility is that the ring actively generates tension along its circumference, which due to the spherical geometry of the embryo gives rise to a force pulling on the EVL margin (supplementary material). To test for the presence of circumferential tension within the YSL actomyosin ring, we used a UV laser cutter (12) to sever the actomyosin network along a 20- μm -long line 20 μm away from the EVL/YSL border in an orientation perpendicular to the EVL margin (Fig. 2A and movie S4). Perpendicular cuts resulted in rapid recoil velocities of the actomyosin network at 60 to 70% epiboly that decayed exponentially over time (12) (6.5 to 7.5 hpf; $v_{\text{cut}} = 19.1 \pm 1.5 \mu\text{m/s}$, $\tau = 1.7 \pm 0.2 \text{ s}$, $N = 40$; fig. S2D). Repeating this experiment at different stages of EVL epiboly revealed that initial recoil

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Fig. 1. Actomyosin ring morphogenesis and function. (A and B) In *Tg(actb1:myl12.1-eGFP)* embryos at 40% epiboly (A), an initially diffuse and broad actomyosin band (orange bar) narrows along the AV axis to form a distinct cable-like structure at 70% epiboly (B). Scale bar, 100 μm . (C) Quantification of myosin-2 peak fluorescence intensity and width of the actomyosin ring during epiboly (supplementary material; fig. S1). Error bars correspond to error of the mean at 95% confidence unless noted otherwise. (D and E) Local disruption of the actomyosin ring in *Tg(actb1:myl12.1-eGFP)* embryos at 60% epiboly by consecutive UV-laser ablation [white dashed rectangle, (D)] reduces advancement of the adjacent EVL margin [dark blue, (E)]. Scale bar, 50 μm . (F) Quantification of advancement rate of the EVL margin adjacent (dark blue) and farther away (light blue) from the ablation site by particle image velocimetry show a $22.6 \pm 6.3\%$ reduction. $P = 0.000015$.

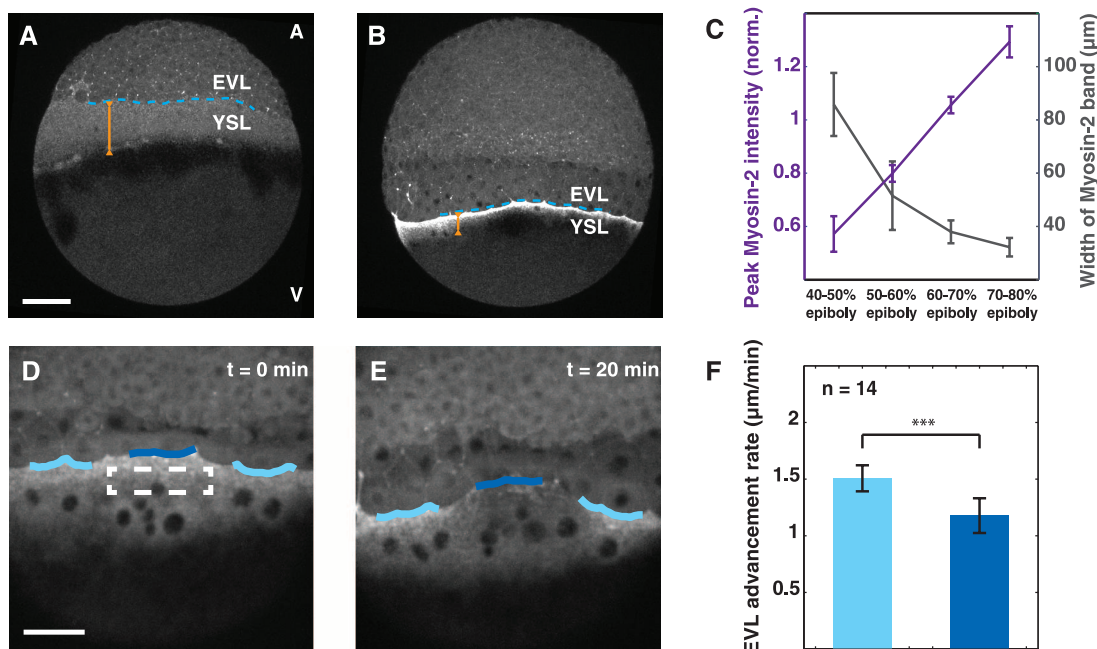


Fig. 2. Actomyosin ring tension and flow. **(A)** *Tg(actb1:myl12.1-eGFP)* embryos at 65% epiboly where the actomyosin ring was cut along a 20- μ m line perpendicular (red) and parallel (green) to the EVL margin. Scale bars, 10 μ m. **(B)** Initial laser cut recoil velocities at different stages of epiboly for perpendicular (red) and parallel cuts (green) as obtained by exponentially fitting the quantified recoil velocity curves (fig. S2, A to E). Error bars represent the 95% confidence interval of the fit result. **(C)** Cortical flows within the actomyosin ring of a *Tg(actb1:myl12.1-eGFP)* embryo injected with *lifeact-RFP* mRNA labeling myosin-2 (left) and F-actin (right), respectively, at 60% epiboly (movie S8). The EVL margin (blue line) and the two-dimensional (2D) vector field of actomyosin flow (yellow arrows) are shown. Scale bar, 20 μ m. **(D)** Average profile of cortical flow in embryos at 60 to 70% epiboly ($N = 22$). Negative velocities correspond to flow toward the animal pole. Inhibiting myosin-2 activity by treatment with blebbistatin reduces recoil velocities for both parallel and perpendicular cuts as well as retrograde flow, indicating that cortical tension and flow are due to myosin-2 activity (fig. S4).

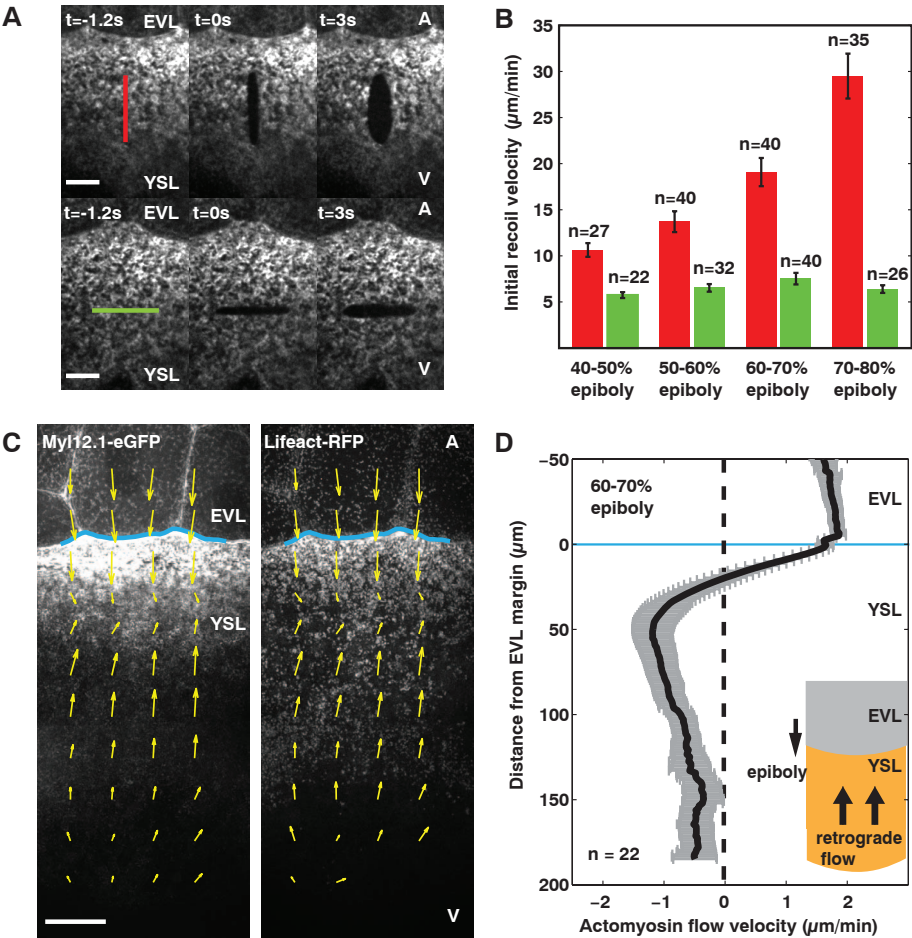
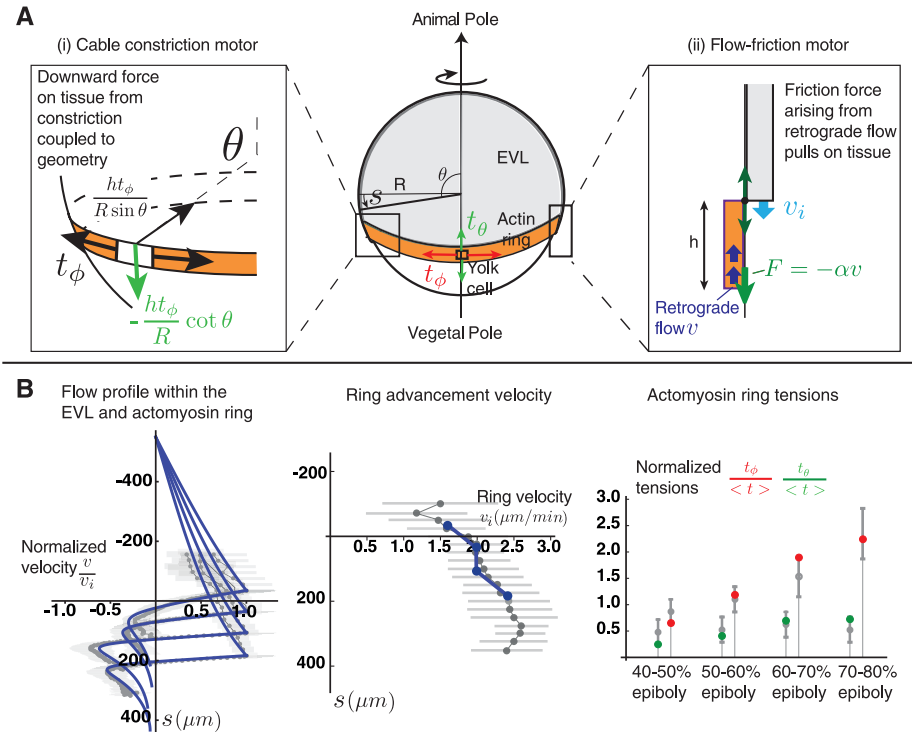


Fig. 3. Theoretical description of EVL epiboly. **(A)** The EVL and actomyosin ring are represented by two thin and compressible viscous layers with an internal active tension, lying on a sphere and mechanically connected at the EVL margin. (i) Circumferential tension within the ring coupled to the embryo curvature moves the EVL toward the closest pole. (ii) Myosin-dependent retrograde flow coupled to friction against an underlying substrate generates a pulling force on the EVL. **(B)** Global fit of the model predictions for flows (blue curves, left), ring advancement velocity (blue curve, middle), and tensions (green and red dots, right) compared to the experimentally measured EVL and actomyosin ring flow profiles, and actomyosin ring relative tensions obtained from laser ablation (gray curves and dots in the respective panels) at successive epiboly stages. Experimental data are fitted to Eqs. 42 to 46 in the supplementary material. Tensions are normalized to the average ring tension ($\langle t \rangle$). The fitting procedure yields the hydrodynamic length $l = 76 \pm 10 \mu\text{m}$ indicative of nonzero friction exerted on the actomyosin ring.



velocities increase over the course of gastrulation (Fig. 2B), suggesting that circumferential tension within the actomyosin ring becomes larger during EVL epiboly progression.

Because of the spherical geometry of the yolk cell, circumferential active tension will result in a net force pulling on the EVL margin, which is zero when the ring is at the equator and grows as the ring approaches the pole (Fig. 3A and supplementary material). Consequently, when the ring is near the equator, tension in the animal-vegetal (AV) direction within the ring is expected to be much smaller than circumferential tension (supplementary material). A detailed calculation that considers the force balance of a constricting ring positioned on a sphere and connected to the EVL at one end shows that AV tension scales as $h/R \cot \theta$, with θ being the polar angle, h the width of the ring, and R the radius of the yolk cell. As a result, AV tension is expected to increase during epiboly progression and reach at most ~20% of circumferential tension at 80% epiboly (supplementary material). We tested this assumption by cutting the actomyosin network along a 20- μ m-long line 20 μ m away from the EVL/YSL border in an orientation parallel to the EVL margin (Fig. 2A and movie S5). Contrary to our expectations, we found that the initial recoil velocity of the actomyosin network for parallel cuts was approximately half of the initial recoil velocity for perpendicular cuts at 60 to 70% epiboly ($v_{\text{cut}} = 7.5 \pm 1.0 \mu\text{m/s}$, $N = 40$) and remained constant during epiboly pro-

gression (fig. S2D and Fig. 2B). Notably, characteristic decay times were similar for both parallel and perpendicular cuts and at different stages during epiboly (fig. S2, B to E), suggesting that material properties of the actomyosin network are constant and isotropic. We conclude that AV tension is much larger than expected if the actomyosin ring were acting as a constricting ring only.

To elucidate where the additional tension in the AV direction comes from, we analyzed the dynamic distribution of actin and myosin-2 within the YSL at high spatial and temporal resolution using spinning disk confocal microscopy. Formation of the actomyosin ring was accompanied by flows of actin and myosin-2 from vegetal parts of the YSL toward the margin of the EVL (Fig. 2C and movie S8). These retrograde actomyosin flows were slow (0.3 $\mu\text{m/min}$) at the onset of EVL epiboly (40% epiboly), but increased their retrograde velocity as epiboly proceeded, reaching maximal flow velocities of 1.2 $\mu\text{m/min}$ around 70% epiboly stage (Fig. 2D and fig. S3).

To investigate if retrograde flows within the YSL could give rise to additional tension along the AV axis of the YSL actomyosin network, we developed a theoretical description of YSL actomyosin network dynamics within the framework of thin films of active fluids (12–14) (supplementary material). Cortical flows have previously been associated with gradients of actomyosin contractility along the direction of flow (12, 15). We considered the actomyosin ring to be an isotropic

thin-film fluid with myosin-2-dependent active tension (Fig. 3 and supplementary material). The animal side of the ring is mechanically connected to the EVL (4), and tension and velocity are continuous at the interface between the two layers (Fig. 3A). On the vegetal side of the ring, myosin-2 density, and consequently active tension, gradually decreases toward zero, consistent with tension measurements at the vegetal pole (fig. S2F and movie S9). This active tension gradient can give rise to contractile flow (Fig. 3 and supplementary material). The ring here is in a “stationary state,” where flow within the ring is compensated and balanced by continuous actomyosin turnover and regrowth (fig. S5), reminiscent of situations in crawling cells where retrograde actin flow is compensated by continuous polymerization at the leading edge (16). Turnover also endows the material with fluid character (12, 17).

Applying this model to EVL epiboly, we revealed two contributions to the total force exerted by the ring upon the EVL—one from circumferential tension coupling to geometry, and one from flow. In the absence of friction between the actomyosin ring and adjacent components of the yolk cell, total force on the EVL arises solely through a ring-contraction mechanism that couples to the spherical geometry of the yolk cell (termed “cable-constriction motor”; Fig. 3A). Notably, this cable-constriction motor can drive epiboly only once the actomyosin ring has passed the equator (Fig. 3A and fig. S11). By contrast, when

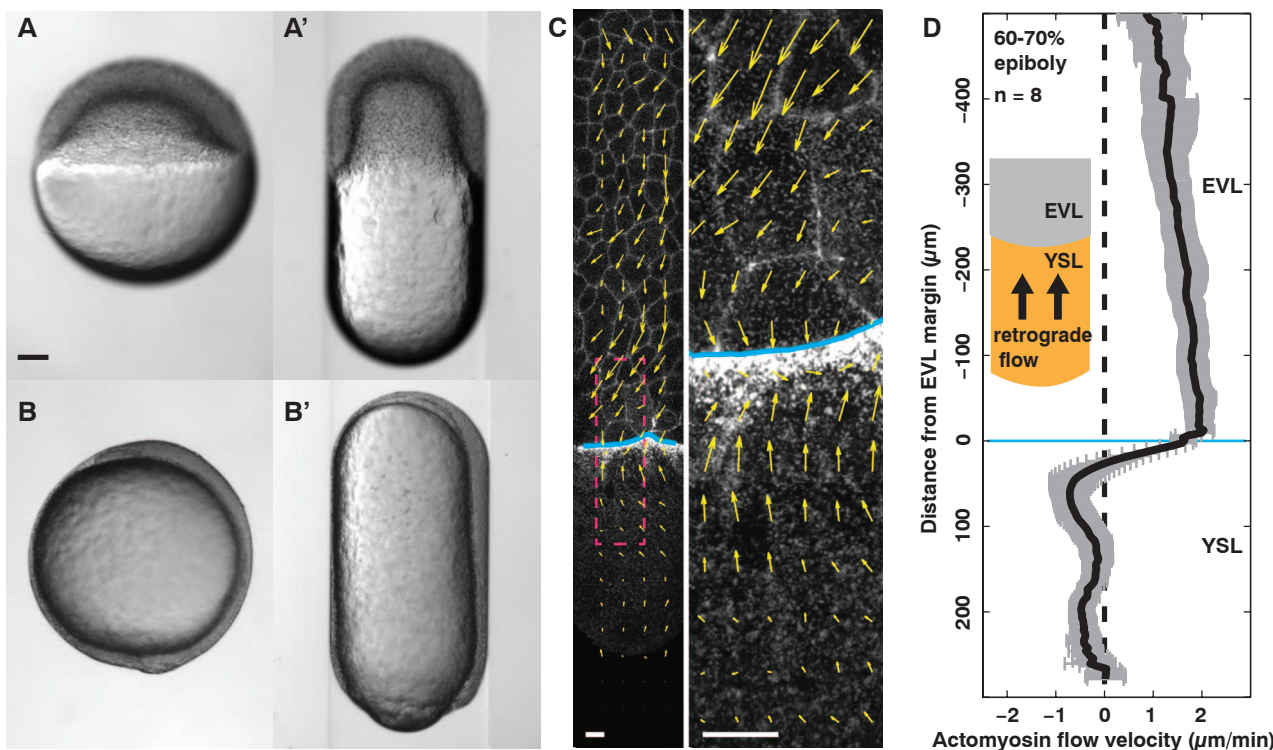


Fig. 4. EVL epiboly without a cable-constriction motor. (A and B) *Tg(actb1:GFP-utrCH)* embryos labeling F-actin aspirated into cylindrical agarose tubes (diameter = 500 μm) at 2.5 hpf. Brightfield images of cylindrical embryos when control embryos were at 30% epiboly (A) and 100% epiboly (B). Scale bar, 100 μm . (C) EVL margin

(blue line) and the 2D vector field of cortical flow (yellow arrows) in cylindrical *Tg(actb1:GFP-utrCH)* embryos at 60% epiboly. Scale bar, 25 μm . (D) Average actomyosin flow profiles of cylindrical embryos at 60 to 70% epiboly within the EVL and YSL. Negative velocities correspond to flow toward the animal pole.

friction is present, total force on the EVL also has a geometry-independent contribution, where retrograde flow of actin and myosin-2 is resisted by friction (termed “flow-friction motor”; Fig. 3A). In the embryo, friction will arise when the flow velocity in the ring is different from the velocity of the adjacent material, such as the yolk cell plasma membrane and the yolk cytoplasm. Consistent with this, we observed differential flow velocities between the actomyosin in the ring and adjacent microtubules within the YSL (fig. S6). This flow-friction motor pulls the EVL in a direction opposite to the actomyosin flow, operates at any stage, and can drive epiboly before passing the equator (Fig. 3A and fig. S11). Notably, friction-resisted flow provides additional tension in the AV direction, consistent with the small degree of tension anisotropy observed in the laser ablation experiments (Fig. 2B). Furthermore, experimentally measured flow profiles within the EVL and the actomyosin ring, as well as the relative tensions obtained from laser ablation, are accurately predicted by our theoretical description at all stages when friction against the substrate is taken into account (Fig. 3B). To conclude, we identified two distinct modes of ring propulsion: a cable-constriction motor due to circumferential contraction of the YSL actomyosin network, and a flow-friction motor due to contraction along the AV axis of the network.

We next asked if the flow-friction motor is sufficient to drive EVL epiboly. To this end, we took advantage of the predicted geometry dependence of the cable-constriction motor. Because the cable-constriction motor cannot exert a net force on the EVL when positioned right at the equator, propulsion by this motor would be hindered when the yolk cell is deformed from its original spherical geometry into a cylindrical shape.

We thus deformed the yolk cell into a cylindrical shape by aspirating pre-gastrula-stage embryos (2.5 hpf) into agarose tubes of a diameter smaller than that of the embryo and analyzed resulting changes in EVL movements. To verify that the actomyosin ring is unperturbed in cylindrical embryos, we analyzed the distribution and flow of actin and myosin-2 within the YSL of cylindrical embryos. We found that both the accumulation of actin and myosin-2 in a ring-like structure adjacent to the EVL/YSL border and their retrograde flows from the vegetal pole toward the EVL/YSL border were largely unaffected in cylindrical embryos as compared to normal-shaped control embryos (Fig. 4 and movie S10). This suggests that the actomyosin ring remains intact in cylindrical embryos. We observed that EVL movements were largely unaffected in cylindrical embryos and proceeded with velocities similar to those of spherical control embryos ($2.0 \pm 0.2 \mu\text{m}/\text{min}$ compared to $1.9 \pm 0.1 \mu\text{m}/\text{min}$ at 60 to 70% epiboly; compare Figs. 4D and 2D). This shows that the cable-constriction motor is not essential for EVL epiboly movements and indicates that the flow-friction motor is sufficient to drive this process.

Our findings have major implications for the function of actomyosin rings in morphogenesis. Whereas the prevalent model of actomyosin ring function assumes circumferential contraction as the main force-generating process, we present evidence that friction-resisted actomyosin flows can represent an equally important process mediating ring function. This raises the possibility of a more general role of cortical flows in morphogenetic pattern formation processes (18).

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Acknowledgments: We are grateful to M. Sixt, T. Bollenbach, and E. Martin-Blanco for advice and the service facilities of the IST Austria and MPI-CBG for continuous help. M.B., G.S., S.W.G., and C.-P.H. synergistically and equally developed the presented ideas and the experimental and theoretical approaches. M.B. and P.C. performed the experiments; G.S. developed the theory; and R.H., F.O., and J.R. contributed to the experimental work. This work was supported by a grant from the Fonds zur Förderung der wissenschaftlichen Forschung (FWF) and the Deutsche Forschungsgemeinschaft (DFG) (I930-B20) to C.-P.H., S.W.G., and G.S.

Supplementary Materials

www.sciencemag.org/cgi/content/full/338/6104/257/DC1
Supplementary Text
Figs. S1 to S16
Materials and Methods
References (19–36)
Movies S1 to S10

1 May 2012; accepted 10 September 2012
10.1126/science.1224143

Photomechanical Responses in *Drosophila* Photoreceptors

Roger C. Hardie* and Kristian Franze

Phototransduction in *Drosophila* microvillar photoreceptor cells is mediated by a G protein-activated phospholipase C (PLC). PLC hydrolyzes the minor membrane lipid phosphatidylinositol 4,5-bisphosphate (PIP₂), leading by an unknown mechanism to activation of the prototypical transient receptor potential (TRP) and TRP-like (TRPL) channels. We found that light exposure evoked rapid PLC-mediated contractions of the photoreceptor cells and modulated the activity of mechanosensitive channels introduced into photoreceptor cells. Furthermore, photoreceptor light responses were facilitated by membrane stretch and were inhibited by amphipaths, which alter lipid bilayer properties. These results indicate that, by cleaving PIP₂, PLC generates rapid physical changes in the lipid bilayer that lead to contractions of the microvilli, and suggest that the resultant mechanical forces contribute to gating the light-sensitive channels.

In most invertebrate photoreceptor cells, the visual pigment (rhodopsin) and other components of the phototransduction cascade are localized within tightly packed microvilli (tubular membranous protrusions), together forming a light-guiding rod-like stack (rhabdomere; Fig. 1). After photoisomerization, rhodopsin activates a heterotrimeric guanine nucleotide-binding protein

(Gq protein), releasing its guanosine triphosphate-bound α subunit, which in turn activates phospholipase C (PLC; Fig. 1C). How PLC activity leads to gating of the light-sensitive transient receptor potential channels (TRP and TRPL) in the microvilli is unresolved (1–3). PLC hydrolyzes the minor membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PIP₂), yielding soluble inositol

1,4,5-trisphosphate (InsP₃), diacylglycerol (DAG, which remains in the inner leaflet of the microvillar lipid bilayer), and a proton. The light-sensitive channels in *Drosophila* photoreceptors can be activated by a combination of PIP₂ depletion and protons (4), but it remains unclear how PIP₂ depletion might contribute to channel gating. It has been speculated that changes in membrane properties play a role (4, 5), and members of the TRP ion channel family have been repeatedly, although controversially, implicated as mechanosensitive channels (6, 7). This led us to ask whether cleavage of the bulky, charged inositol head group of PIP₂ (Fig. 1C) from the inner leaflet might alter the physical properties of the lipid bilayer in the microvilli, resulting in mechanical forces that contribute to channel gating.

Remarkably, *Drosophila* photoreceptors responded to light flashes with small (<1 μm) but rapid contractions that were directly visible in

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Fig. 1. AFM measurements of photomechanical responses. **(A)** An AFM cantilever contacts distal tips of ommatidia in an excised *Drosophila* retina. **(B)** An ommatidium, containing photoreceptors (orange) and pigment cells (red). Elements of the phototransduction cascade are contained within microvillar rhabdomeres (two shown in longitudinal section, seven in cross section), which are rodlike stacks $\sim 80 \mu\text{m}$ in length containing $\sim 30,000$ microvilli. Right: Electron micrograph cross section of a rhabdomere (scale bar, $1 \mu\text{m}$), showing tubular microvilli, each $\sim 50 \text{ nm}$ in diameter, with lumen in diffusional continuity with the cell body. **(C)** Phototransduction cascade. Rhodopsin (R) is photoisomerized to metarhodopsin (M^*), which catalyzes release of the Gq protein α subunit to activate PLC. PLC hydrolyzes PIP_2 (red), leaving DAG (green) in the membrane. Ca^{2+} influx via TRP channels inhibits PLC. **(D)** Lower traces: AFM measurements of contractions (cantilever z-position) in a wild-type retina in response to 5-ms flashes, with intensity increased from ~ 200 to 8000 effectively absorbed photons per photoreceptor. Blue traces: Whole-cell current-clamped voltage responses to the same stimuli recorded from a dissociated photoreceptor cell. **(E)** Contractions evoked by 5-ms flashes covering the full intensity range (~ 200 to 10^6 photons) in a wild-type retina. **(F)** Same on faster time base. **(G)** Response versus intensity (R/I) functions of contractions (nm) from wild-type retinas (mean $\pm$ SEM, $N = 13$), and peak voltage (mV) recorded from dissociated photoreceptors (blue; means $\pm$ SEM, $N = 6$). **(H)** Responses to flashes ($\sim 5 \times 10^4$ photons) in *trpl* mutant before and after (red) channel block by $50 \mu\text{M}$ La^{3+} and $10 \mu\text{M}$ ruthenium red (RR), which prevents inhibition of PLC by Ca^{2+} influx. Blue trace: Lack of response in *norPA*^{P24} (PLC mutant) despite using flashes of higher intensity ($\sim 2 \times 10^5$ photons; $N = 3$). **(I)** R/I function of contractions from *trpl* retinas before and after (red) channel block by La^{3+} and RR (means $\pm$ SEM, $N = 5$). For intensity calibration, see fig. S2.

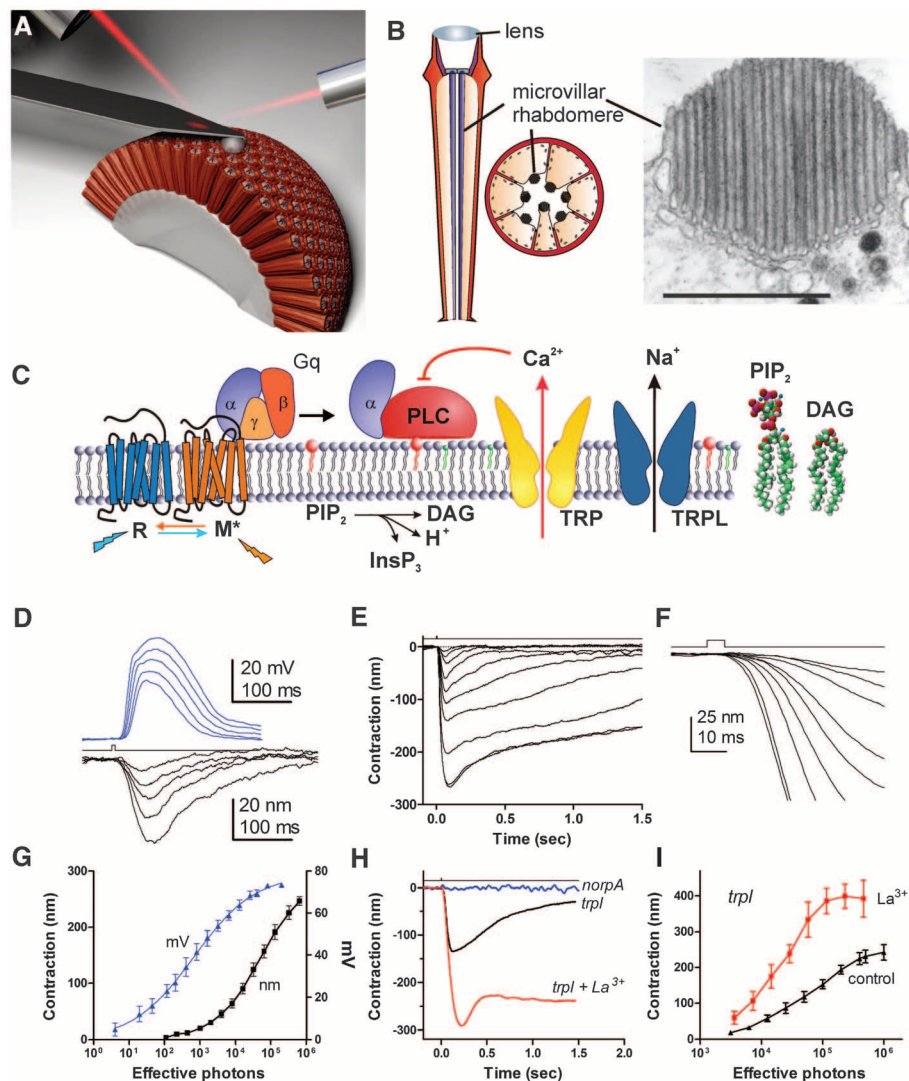
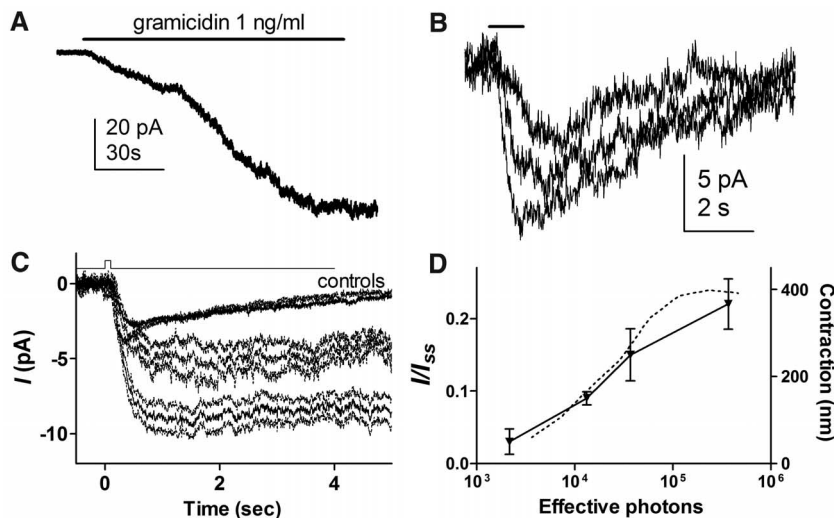


Fig. 2. Light-induced modulation of gramicidin channels. **(A)** Whole-cell recordings from *trpl;trp* mutant photoreceptor cell lacking all native light-sensitive channels. Perfusion with gramicidin induced a constitutive inward current. **(B)** Flashes of increasing intensity (5×10^3 to 4×10^5 effective photons), each 1 s (denoted by bar at upper left), up-regulated the current. **(C)** Averaged responses ($\pm$ SEM) to 100-ms flashes containing 1.3×10^4 (middle trace, $N = 4$) and 3.7×10^4 (lower trace, $N = 10$) effective photons (data pooled from *trpl;trp* and *trpl* mutants recorded in the presence of La^{3+} and RR). The same flashes delivered before gramicidin application (controls) induced residual, noise-free transient currents of uncertain origin. **(D)** R/I function (after subtracting control responses measured before gramicidin perfusion) expressed as a fraction of the steady-state gramicidin current I/I_{ss} (means $\pm$ SEM, $N = 4$). Dotted curve: R/I function of contractions measured by AFM in *trpl* mutant in the presence of La^{3+} and RR, replotted from Fig. 1I.



dissociated cells via bright-field microscopy (see movie S1). To obtain improved temporal and spatial resolution, we recorded these photomechanical responses with an atomic force microscope (AFM) (8), positioning the AFM cantilever on the distal tips of photoreceptors in a whole excised retina glued to a coverslip (Fig. 1A). Contact force (~ 100 pN) was maintained constant, so that changes in sample height resulted in immediate, matching changes in the cantilever's z -position. Contractions were elicited indefinitely by repeated brief flashes of modest intensity, with kinetics similar to those of electrical responses recorded from dissociated photoreceptors (Fig. 1D and fig. S1). The latencies of contractions induced by the brightest stimuli (4.9 ± 0.9 ms, mean $\pm$ SEM, $N = 11$; Fig. 1F) were significantly shorter than the latencies of voltage responses to the same stimuli (6.6 ± 0.6 ms, $N = 6$; $P = 0.002$, unpaired two-tailed t test). Only a few hundred effectively absorbed photons per photoreceptor were required to elicit detectable contractions, which saturated with flashes containing $\sim 10^6$ photons, corresponding to ~ 30 effectively absorbed photons per microvillus (Fig. 1, E and G). This intensity dependence overlapped with that of the electrical response (Fig. 1G and supplementary text). Like the electrical responses, the contractions were eliminated in mutants lacking PLC (*norpA*^{P24}), which shows that they too required PLC activity (Fig. 1H).

Because PLC activity is normally terminated by Ca^{2+} influx through the light-sensitive channels, net PIP_2 hydrolysis is enhanced when the light-sensitive current is blocked (9, 10). We therefore measured contractions in *trpl* mutants expressing only TRP light-sensitive channels before and after blocking TRP channel activity with La^{3+} and ruthenium red (RR). Indeed, after blocking the light-sensitive channels the contractions were enhanced, more sensitive to light, and saturated at lower intensities, corresponding to only ~ 1 to 5 effectively absorbed photons per microvillus (Fig. 1, H and I). In the absence of Ca^{2+} influx, such intensities deplete virtually all microvillar PIP_2 , resulting in temporary loss of sensitivity to light (10). After such saturating flashes, the photomechanical response was also temporarily refractory, recovering sensitivity with a time course ($t_{1/2} \sim 40$ s) similar to that of PIP_2 resynthesis (10). By contrast, without channel blockers, sensitivity recovered within ~ 10 s (fig. S3).

Blockade of all light-sensitive current in these experiments also shows that the contractions cannot result from any downstream effects of Ca^{2+} influx or osmotic changes caused by ion fluxes associated with the light response. Therefore, these results indicate that the contractions result from hydrolysis of PIP_2 . Although we do not exclude other downstream effects of PLC, the speed of the contractions supports a simple and direct mechanism. Cleavage of the bulky head groups from PIP_2 molecules, which represent 1 to 2% of lipids in the plasma membrane, leaves DAG in the membrane, which occupies a substantially smaller area than PIP_2 . This should increase mem-

brane tension, leading to shrinkage of the microvillar diameter, as reported for the action of PLC on the diameter of artificial liposomes (11). Integrated over the stack of $\sim 30,000$ microvilli, such a mechanism seems capable of accounting for the observed macroscopic contractions, which represent at most $\sim 0.5\%$ of the rhabdomere length (see supplementary text). Within each microvillus, we suggest that the alteration to the mechanical properties of the lipid bilayer may contribute to channel gating.

To test whether phototransduction generates sufficient mechanical forces to gate mechanosensitive channels (MSCs), we made whole-cell patch-clamp recordings from dissociated photoreceptors lacking all light-sensitive channels (*trpl*;*trp* double mutants, or *trpl* mutants exposed to La^{3+} and RR). We then perfused the photoreceptors with gramicidin, a monovalent (Ca^{2+} -impermeable) cation channel and one of the

best-characterized MSCs, which is known to be regulated by changes in bilayer physical properties (12, 13). Incorporation of gramicidin channels into the membrane generated a constitutive inward current that stabilized after a few minutes (Fig. 2A). Despite having replaced the native light-sensitive channels with MSCs, the photoreceptors still responded to light, with a rapid increase in the gramicidin-mediated current (Fig. 2, B and C). Like the photomechanical responses recorded after blocking Ca^{2+} influx through the light-sensitive channels (Fig. 1H), these gramicidin-mediated responses inactivated slowly (Fig. 2C), were temporarily refractory to further stimulation and had a similar intensity dependence (Fig. 2D).

To test whether the light-sensitive channels were mechanically sensitive, we manipulated membrane tension osmotically. Channels were not directly activated by perfusing cells with hyper- or hypo-osmotic solutions; however, we reasoned

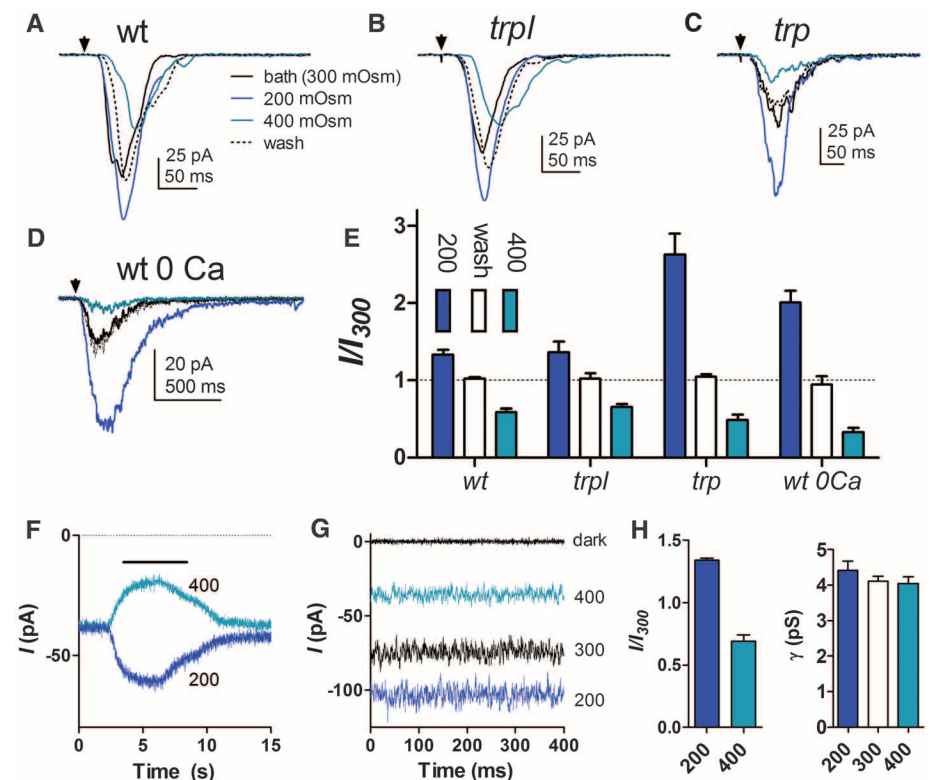


Fig. 3. Modulation of light-sensitive channels by osmotic pressure. (A to D) Whole-cell voltage-clamped responses to 1-ms flashes (~ 50 effective photons) in control bath (300 mOsm) were reversibly increased by perfusion with 200 mOsm solution and suppressed by 400 mOsm in wild-type (A), *trpl* (B), and *trp* (C) mutants as well as in wild-type photoreceptors recorded in Ca^{2+} -free solutions (D). (E) Response amplitudes (I/I_{300}) after hyperosmotic (400 mOsm) and hypo-osmotic (200 mOsm) challenges normalized to control responses in 300 mOsm bath. Data are means $\pm$ SEM; $N = 4$ to 8 cells. All conditions plotted were significantly increased (200 mOsm) or decreased (400 mOsm) relative to control responses from the same cells [$P < 0.005$; analysis of variance (ANOVA) followed by posttest for trend]. (F) Spontaneous TRP channel activity (from *trpl* mutant) after several minutes of recording, using pipettes lacking nucleotide additives. Perfusion with 400 or 200 mOsm (bar) reversibly suppressed and facilitated this “rundown current” (RDC). (G) Channel noise resolved on a faster time base, plus trace recorded in dark before onset of RDC. (H) Left: Amplitude of steady-state RDC normalized to value at 300 mOsm. Right: Effective single-channel conductance (γ) estimated by variance/mean ratio (means $\pm$ SEM, $N = 7$). Although macroscopic RDC was substantially modulated ($P < 0.001$; ANOVA, posttest for trend), single-channel conductance was not significantly affected by osmotic manipulation ($P > 0.2$).

that it would be impossible to mimic the exact physical effects of PIP₂ hydrolysis, which would include a specific combination of changes in membrane tension, curvature, thickness, lateral pressure profile, charge, and pH. We therefore tested whether osmotic manipulation could enhance or suppress light-sensitive channel activity. In wild-type photoreceptors, light-induced currents were rapidly and reversibly facilitated by ~50% after perfusion with hypo-osmotic solutions (200 mOsm), which, like PIP₂ depletion, would be expected to alleviate crowding between phospholipids, increase tension, and reduce membrane thickness. Conversely, responses in hyperosmotic (400 mOsm) solutions were about half those in control solutions (Fig. 3). Analysis of single-photon responses (quantum bumps) indicated that modulation resulted from changes in both quantum efficiency (fraction of rhodopsin photoisomerizations generating a quantum bump) and bump amplitude (fig. S4 and supplementary text). Recordings from *trp* and *trpl* mutants showed that both TRP and TRPL channels were modulated, although facilitation of currents mediated by TRPL channels (in *trp* mutants) was more pronounced (Fig. 3). Modulation of the light response by osmotic manipulation was at least as pronounced in Ca²⁺-free bath (Fig. 3D), indicating that facilitation by membrane stretch did not result from leakage of Ca²⁺ into the cell from the extracellular space.

To test whether modulation might have been mediated by effects on upstream components of the cascade such as PLC (14), we measured the activity of spontaneously active TRP channels in recordings made with pipettes lacking adenosine triphosphate. Under these conditions, PIP₂ becomes depleted (thereby removing PLC's substrate), sensitivity to light is lost, and the TRP channels enter a constitutively active ("rundown") state uncoupled from the phototransduction cas-

cade (15, 16). Nonetheless, the channels were still similarly modulated by osmotic manipulation, whereas single-channel conductance, estimated by noise analysis, was unaffected (Fig. 3, F to H). These results indicate that osmotic pressure directly modulated the open probability of both TRP and TRPL channels.

MSCs such as gramicidin are sensitive to amphiphilic compounds, which insert into the lipid bilayer. Because they are attracted to anionic phospholipids, cationic amphipaths insert preferentially into the inner leaflet, where they increase crowding, promote negative (concave) curvature, and decrease membrane stiffness (17, 18). We found that four structurally unrelated cationic amphipaths were all effective, reversible inhibitors of the light-induced current. Neither light-induced PLC activity (measured using a genetically targeted PIP₂-sensitive biosensor to monitor PIP₂ hydrolysis) nor single-channel conductance were substantially affected (fig. S5). The 50% inhibitory concentrations (IC₅₀ values) were much higher than those of their traditional drug targets and ranged over approximately three orders of magnitude. However, after correcting for pK_a and partitioning, the effective concentration of the compounds in the membrane was similar (~5 mM) in each case (Fig. 4). Thus, their mode of action is likely related to their physicochemical properties rather than conventional drug-receptor interactions. Because cationic amphipaths are also lipophilic weak bases, and because we propose that protons are also critical for activating the light-sensitive channels (4), an alternative but not mutually exclusive possible mechanism of action is as lipophilic pH buffers of the membrane environment. We also note that polyunsaturated fatty acids (PUFAs) such as arachidonic and linolenic acid—which are effective activators of both TRP and TRPL (5, 19)—are not only anionic amphipaths (predicted to have opposite effects

to cationic amphipaths) but also, as weak acids, natural protonophores; such a dual action could account for their agonist effect.

The mechanism of activation of the light-sensitive channels in invertebrate microvillar photoreceptors has long remained an enigma (2, 3, 20). Neither InsP₃ nor DAG—the two obvious products of PIP₂ hydrolysis—are reliable agonists for the light-sensitive channels. Although PUFAs are effective agonists and might be generated from DAG, a DAG lipase with the appropriate specificity has not been found in the photoreceptors (21). By contrast, two neglected consequences of PLC activity—the depletion of its substrate (PIP₂) together with protons released by PIP₂ hydrolysis—were recently shown to potentially activate the light-sensitive channels in a combinatorial manner (4). Our results support the hypothesis that the effect of PIP₂ depletion is mediated mechanically by changes to the physical properties of the lipid bilayer, thereby introducing the concept of mechanical force as an intermediate or "second messenger" in metabotropic signal transduction.

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Acknowledgments: We thank D. G. Stavenga, S. B. Laughlin, and M. Postma for comments on the manuscript; O. Andersen for advice on the use of gramicidin; and J. Grosche (Effigios AG) for artwork for Fig. 1A. Supported by UK Biotechnology and Biological Sciences Research Council grant BB/G0068651 (R.C.H.) and a UK Medical Research Council Career Development Award (K.F.). Author contributions: project initiation, R.C.H.; whole-cell electrophysiology experiments, R.C.H.; AFM measurements, K.F., R.C.H.; paper written by R.C.H. with contribution from K.F.

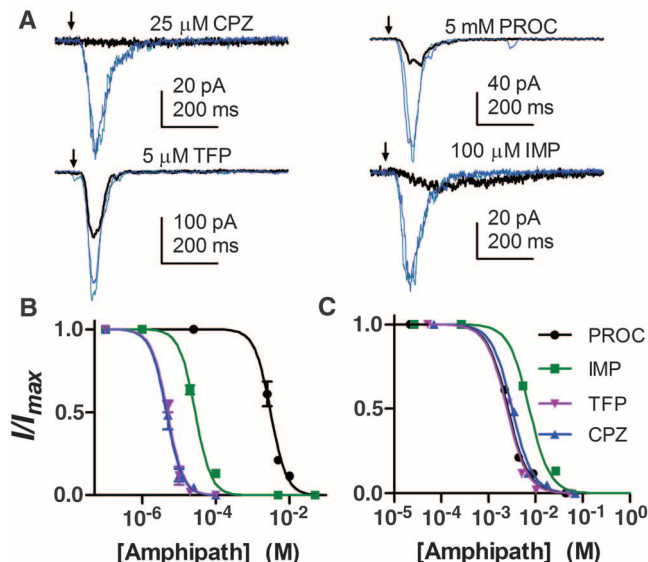
Supplementary Materials

www.sciencemag.org/cgi/content/full/338/6104/260/DC1
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26 March 2012; accepted 7 August 2012
10.1126/science.1222376

Fig. 4. Cationic amphipaths

inhibit the light response. (A) Response to 1-ms flashes in *trp* mutants in the presence of procaine (PROC), imipramine (IMP), trifluoperazine (TFP), and chlorpromazine (CPZ). Control responses before (turquoise) and after washout (blue) are superimposed. (B) Dose response functions (means ± SEM: PROC, *N* = 4; IMP, *N* = 3; TFP, *N* = 3; CPZ, *N* = 6) fitted by inverse Hill curves (slope constrained at *n* = 2), based on raw values (IC₅₀: PROC, 3.1 mM; IMP, 27 μM; TFP, 4.9 μM; CPZ, 4.5 μM). (C) Same as in (B) after correction for pK_a and octanol partition coefficients, reflecting predicted concentration in the lipid membrane (IC₅₀: PROC, 2.7 mM; IMP, 7 mM; TFP, 3.4 mM; CPZ, 2.3 mM).



Bacterial Quorum Sensing and Metabolic Incentives to Cooperate

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The opportunistic pathogen *Pseudomonas aeruginosa* uses a cell-cell communication system termed “quorum sensing” to control production of public goods, extracellular products that can be used by any community member. Not all individuals respond to quorum-sensing signals and synthesize public goods. Such social cheaters enjoy the benefits of the products secreted by cooperators. There are some *P. aeruginosa* cellular enzymes controlled by quorum sensing, and we show that quorum sensing–controlled expression of such private goods can put a metabolic constraint on social cheating and prevent a tragedy of the commons. Metabolic constraint of social cheating provides an explanation for private-goods regulation by a cooperative system and has general implications for population biology, infection control, and stabilization of quorum-sensing circuits in synthetic biology.

A cyl-homoserine lactone (AHL) quorum sensing is common among *Proteobacteria*. This is a form of cell-cell communication that allows bacteria to monitor their population density and activate specific sets of genes when populations have reached a threshold density (1–3). Quorum sensing controls the production of a variety of public goods, functions shared

by all individuals in the group. Experiments with *P. aeruginosa* support the view that quorum sensing controls cooperativity between *P. aeruginosa* cells (4–6). AHL signaling in *P. aeruginosa* is complex and involves multiple signals and receptors (7). A key system involves LasI, which generates the diffusible AHL signal *N*-3-oxododecanoylhomoserine lactone

(C12-HSL) and LasR, a C12-HSL-responsive transcription activator (8). Together C12-HSL and LasR directly activate dozens of genes (9), with a heavy overrepresentation of genes coding for extracellular factors or production of extracellular factors, although there are some LasR-activated genes coding for cellular enzymes. Cellular enzymes are not shared among individuals so we call them private goods (10). By contrast, LasR-activated extracellular proteases are known as public goods (5). Growth of *P. aeruginosa* with casein or bovine serum albumin as the sole source of carbon and energy requires these proteases and encourages the emergence of LasR-mutant social cheaters (4, 5), which do not incur the cost of expressing quorum-controlled functions but benefit from the public goods produced by other members of the group. These cheaters have the potential to cause a “tragedy of the commons” wherein the population will be overtaken by

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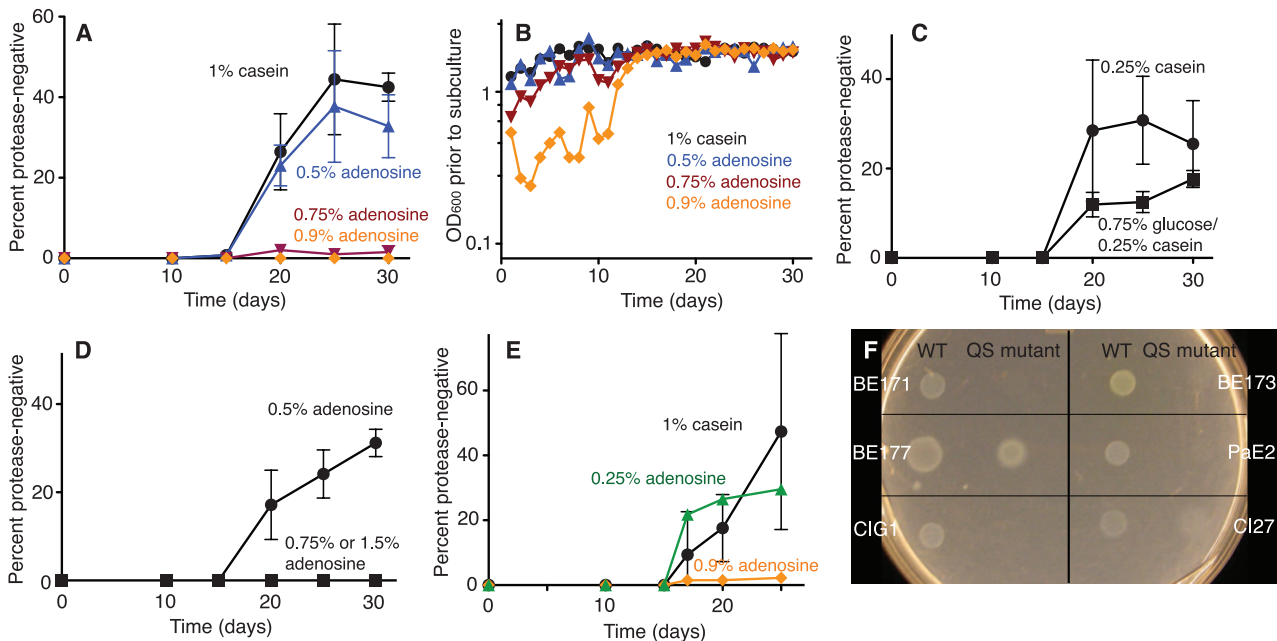


Fig. 1. Adenosine restrains emergence of social cheaters in casein medium. (A) *P. aeruginosa* PAO1 in minimal medium containing casein alone or casein and adenosine at increasing levels of adenosine (total of casein and adenosine is 1% w/v). Protease-negative cheater abundance was determined at 5-day intervals and was essentially identical to the cheater abundance determined by absence of growth on adenosine agar. (B) Growth yields at 24-hour intervals measured as optical density at 600 nm (OD₆₀₀). The cell densities (OD₆₀₀) after 30 days were 1.84 in casein, 1.93 in 0.75% casein with 0.25% adenosine, 1.99 in 0.5% casein with 0.5% adenosine, 1.81 in 0.25% casein with 0.75% adenosine, and 1.89 in 0.1% casein with 0.9% adenosine. The total number of generations in all the experimental groups was nearly equal after 30 days. As discussed in the text, variants with improved growth on adenosine emerged and were isolated from day 25 cul-

tures. (C) The reduction in LasR mutants is not a consequence of resource denial or substitution. Cheaters emerge when strain PAO1 is grown on 0.25% casein with or without 0.75% glucose in place of adenosine. (D) Cheater emergence is restrained when casein is at a fixed concentration and adenosine concentration exceeds 0.5%. (E) The clinical isolate CI27 was grown in minimal medium containing casein or casein and adenosine [as in (A) for strain PAO1]. (F) Quorum sensing is required for growth of most, but not all, *P. aeruginosa* isolates tested. We grew six different *P. aeruginosa* strains (left) and their respective *lasI*, *rhlI* mutants (right) on adenosine agar as described in the methods. In all cases, the wild-type strains grew well. With the exception of BE177, the mutants did not show appreciable growth on adenosine. Except for panels (B) and (F), which are representative experiments, panels show means $\pm$ SEM for three independent experiments.

defectors and ultimately be unable to avail itself of the protein growth substrate (4, 11, 12). Quorum sensing also controls expression of a few private goods. Thus, we asked whether quorum control of such private goods might control social cheating in groups of cooperators.

Microarray analyses (9) suggest that quorum-sensing control of private goods is relatively rare. We used BIOLOG phenotype arrays to confirm this. We compared growth of a *P. aeruginosa* AHL synthesis mutant plus or minus added AHLs on 190 soluble carbon and energy sources and 380 soluble nitrogen sources. Growth was improved in the presence of signal with Glu-Tyr, His-Trp, Tyr-Gly-Gly, Tyr-Phe, Tyr-Trp, and Tyr-Tyr as nitrogen sources, and inosine and D-trehalose as carbon sources (tables S1 and S2). Quorum sensing-dependent growth on inosine was predicted from previous studies showing expression

of the cytoplasmic enzyme nucleoside hydrolase gene (*nuh*) (9) and growth on adenosine was LasR-dependent (13, 14).

Because there is a literature on quorum sensing control of adenosine metabolism we focused our subsequent experiments on whether the obligate pairing of protease secretion (a public good) and adenosine metabolism (a private good) might restrict the ability of social cheaters to invade the population. As described previously (5), long-term growth achieved by daily culture transfer (about 100 generations at about 6 generations per day) with casein as the sole carbon source results in the emergence of a substantial population (about 40%) of putative LasR mutants (Fig. 1A); these individuals do not exhibit extracellular protease activity and cannot grow on adenosine agar. We sequenced *lasR* in 13 protease-negative, adenosine growth-negative isolates

from three independent experiments, and consistent with previous analyses (4, 5), we found LasR-null mutations in every case (C221T, G541A, and C683T, all of which have a LasR-null phenotype).

To test our hypothesis that LasR regulation of a private good constrains the emergence of social cheaters, we grew *P. aeruginosa* with varying concentrations of adenosine plus casein such that, together, the total available carbon source was 1% (w/v). When adenosine was more than 50% of the total, protease-negative, adenosine growth-negative mutants never exceeded 0.2% of the total population (Fig. 1A). Growth of wild-type *P. aeruginosa* on adenosine alone was slow (doubling time of 10 to 12 hours compared with 4 hours on casein) (fig. S1). At the start of an experiment, growth on 50% adenosine, where cheaters eventually emerge, is similar to that on 75% adenosine where cheaters are suppressed (Fig. 1B). Although growth rates vary by condition and day (fig. S1), by day 25, cultures grown with either 50 or 75% adenosine reached a higher density than at the beginning of the experiment (Fig. 1B). We determined the doubling times for cultures grown on 0.25% casein and 0.75% adenosine to be 4 hours at day 1 and 3 hours at day 25. Note that isolates from a day-25 culture grow well with adenosine as the sole carbon and energy source (fig. S1), and although they show normal protease production on skim milk agar plates, these isolates do not grow well in liquid medium with casein as the sole carbon and energy source. The cost of good growth on adenosine appears to be a loss of ability to grow on casein alone. We do not know the genetic basis or evolutionary significance of this finding.

Our hypothesis is that control of adenosine catabolism by quorum sensing serves as a means to suppress cheating. An alternate hypothesis consistent with the results shown in Fig. 1A is that metabolism of any private good can suppress cheating. To test this alternate hypothesis we replaced adenosine with D-glucose, which is metabolized by cellular enzymes of the hexose monophosphate pathway in a manner independent of quorum sensing. Even when glucose constituted 75% of the available carbon, cheaters emerged and reached a substantial proportion of the population (Fig. 1C), which shows that linkage of public and private goods through quorum sensing is required for cheater control.

One might argue that because casein levels were reduced as adenosine levels were increased in the experiments above, cheater suppression results from resource limitation. We addressed this concern in two ways. First, we showed that cheaters emerge in a medium with low levels of casein (0.25%) in the absence of adenosine (Fig. 1C), whereas they do not with 0.25% casein and 0.75% adenosine (Fig. 1A). Second, we asked whether the emergence of LasR mutants would be decreased when the concentration of casein was a constant 0.5% and the concentration of adenosine was the sole variable. We used three concentrations

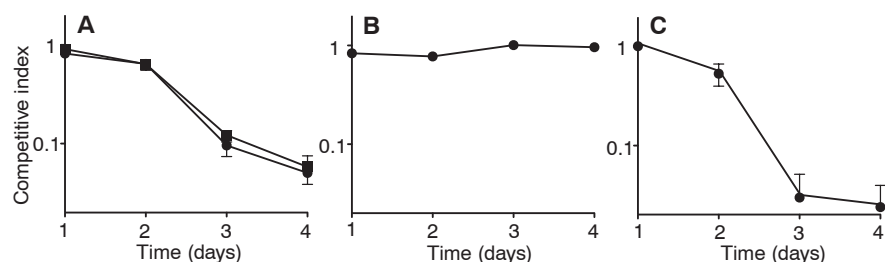


Fig. 2. Adenosine reduces the relative fitness of LasR mutants. (A) Cheaters are rapidly eliminated from adenosine-enriched medium. A mixture of cheaters and wild-type bacteria derived from either strain PAO1 (squares) or C127 (circles) growing on casein alone was transferred to 0.9% adenosine and 0.1% casein and subcultured daily. The abundance of protease-negative bacteria was assessed on skim milk agar. (B) There is no selection against cheaters in LB broth. A mixture of cheaters and wild-type bacteria derived from strain PAO1 was grown in LB broth buffered with MOPS, pH 6.8, with daily subculture, and protease-negative bacteria were counted daily. (C) The addition of glucose, which is metabolized by a quorum sensing-independent pathway, does not prevent adenosine suppression of social cheaters. A wild-type and cheater mixture derived from strain PAO1 was placed into medium containing 1% glucose, 0.9% adenosine, and 0.1% casein, with daily subculture, and protease-negative bacteria were enumerated daily. In all panels, data are displayed as a competitive index or the end point cheater:wild type ratio compared with the initial ratio on a logarithmic scale. Data are shown as means $\pm$ SEM and reflect the combined results of three independent experiments.

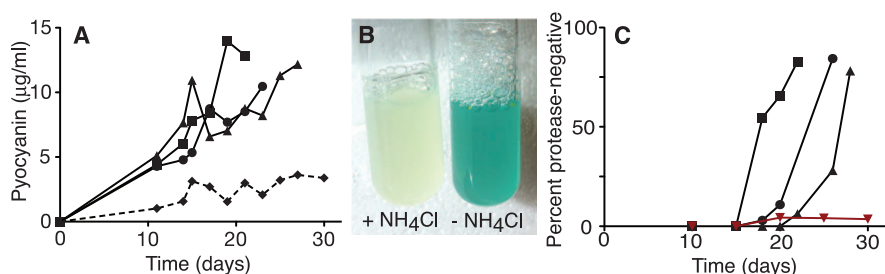


Fig. 3. Quorum-sensing regulation of a private good can prevent a tragedy of the commons. (A) Pyocyanin in cultures of *P. aeruginosa* PAO1 grown with casein as the sole nitrogen, carbon, and energy source (solid lines, three independent experiments). By day 10, there is three to four times as much pyocyanin at the time of transfer as pyocyanin in a culture grown with NH_4Cl as a nitrogen source (dotted line). Pyocyanin was measured as described elsewhere (19). (B) Hyperproduction of the blue phenazine pigment pyocyanin is evident in NH_4Cl -free medium by day 20. (C) Overexpression of quorum sensing-controlled factors correlates with the emergence of LasR mutants, which reach about 80% of the total population, after which cultures do not grow upon transfer. The tragedy of the commons does not occur in a medium containing adenosine (0.75%) and casein (0.25%) (inverted triangles) although LasR mutants are detectable (mean of three experiments; SEM at all points is $<3\%$).

of adenosine: 0.5, 0.75, and 1.5%. As in the experiment described in Fig. 1A, cheater emergence was suppressed if adenosine constituted more than half the available carbon (Fig. 1D).

To ascertain whether suppression of social cheating in media with adenosine is a general characteristic of *P. aeruginosa*, we investigated a quorum sensing–competent isolate from a cystic fibrosis (CF) lung infection. As with the laboratory strain PA01, cheaters emerge after 15 to 20 days of growth on casein, and emergence is suppressed in media with sufficient adenosine (Fig. 1E). Furthermore, we tested *lasI*, *rhlI* mutants deficient in the quorum-sensing signal from six environmental and clinical *P. aeruginosa* isolates (15) and showed that, in all but one case, growth of the mutant on adenosine agar plates was greatly impaired compared with the parent (Fig. 1F). Thus quorum-sensing control of adenosine metabolism is common in *P. aeruginosa* isolates, and adenosine suppression of LasR social cheats is not restricted to PA01. We note that quorum-sensing control of adenosine catabolism is not universal, as the isolate BE177 did not require quorum sensing for growth on adenosine, but adenosine hydrolase is just one of the private goods controlled by quorum sensing in *P. aeruginosa*.

In the previous experiments, social cheaters emerged in populations forced to cooperate by restricting the carbon and energy source to casein. We next asked how rapidly cheaters can be purged from *P. aeruginosa* populations by switching from growth on casein alone to growth on casein plus adenosine. We transferred mixed populations of cooperators and cheaters from day 25 on casein alone to 0.1% casein and 0.9% adenosine. As judged by growth on adenosine agar plates and protease production on skim milk agar plates, there is a strong selection against social cheaters when adenosine is present (Fig. 2A). We also found that when mixed populations of cheaters and cooperators were transferred to Luria-Bertani (LB) broth, a rich growth medium with multiple soluble carbon and energy sources, there was no selection for or against cheaters (Fig. 2B). Thus, neither cheaters nor cooperators have a strong advantage under conditions where public goods are not needed and cooperation is not beneficial.

It seems likely that in many environments where *P. aeruginosa* thrives, multiple carbon and energy sources will be available to this metabolically versatile bacterium. Can adenosine-dependent cheater restraint occur when a solute metabolized by a quorum sensing–independent private good is available? To address this question, we performed an experiment with 0.1% casein, 0.9% adenosine, and 1% glucose, which in itself does not suppress the emergence of cheaters in casein medium (Fig. 1C). The inoculum was a mixed population of cooperators and cheaters that had developed after 25 days on casein alone. We found that the selection against cheaters was at least as strong as that with casein and adenosine only (Fig. 2C).

It is of interest that, in our experiments with casein and those published by others (5), a stable

equilibrium is reached with coexistence of cooperators and cheaters. In these experiments, the cost of cooperation is relatively low (higher than in LB broth but not so high that an equilibrium cannot be reached), and the population of cheaters is limited to roughly 40% of the total. We sought to increase the cost of cooperation by making casein not only the sole source of carbon but also the sole source of nitrogen (by eliminating NH_4Cl as a nitrogen source). We reasoned that by increasing the demand for protease, the cost of cooperation would increase, and our experiments bear this out. Without NH_4Cl , cheaters emerge, and by day 18 to 25, they constitute about 80% of the population. By several measures, public goods, including elastase and phenazines, produced by the cooperators from day 18 to 25 cultures grown in NH_4Cl -free medium are about 10-fold that of the parent (Fig. 3A). One obvious manifestation of the hyperactive quorum-sensing system in cooperators is that, after 18 to 20 days, cultures are visibly blue as a result of copious pyocyanin production (Fig. 3B). Production of this pigment is quorum-sensing regulated (16). We presume the increased cost of cooperation imposed by the nutritional conditions of this experiment leads to a greater relative benefit for cheaters. This results in a rapid emergence of a large cheater population followed by a population collapse, a tragedy of the commons (Fig. 3C).

Can private-goods regulation by quorum sensing protect the population from a tragedy of the commons when the cost of cooperation is relatively high? To address this, we included adenosine with casein in the absence of NH_4Cl . Within the 30-day duration of our experiment there was no population collapse. Cheaters emerged, but they never exceeded 5% (Fig. 3C).

A central tenet of cooperation is that for propagation of a cooperative trait, the group must be as or more successful than the individual, and it must also be (relatively) free of cheaters. Here, we provide an explanation for why bacteria might maintain one or several functions for metabolism by private goods under quorum-sensing control. This explanation is wholly consistent with the view that quorum sensing serves as a communication system for coordination of cooperative behaviors. We believe that co-regulation of functions required for utilization of public and private goods can provide an incentive against cheating. Quorum-sensing control of relatively poorly utilized substrates metabolized by private goods limits the cost of this strategy. LasR regulates private goods other than adenosine hydrolase (9), which should limit the benefit from liberating any single one of these goods from quorum regulation [as in the case of strain BE177 (Fig. 1F)]. It will be of interest to determine whether this type of cheater control is a broadly distributed biological phenomenon or perhaps isolated to *P. aeruginosa* and a few other bacterial species.

Our results provide one plausible explanation for why quorum-sensing mutant social cheaters are not abundant in free-living populations of

P. aeruginosa. Of course, other mechanisms by which evolution of social cheaters might be restricted exist. Dispersal of individuals to new habitats where cooperative behavior is important would select against social cheats. Such a single-cell bottleneck has just been described in the social amoeba *Dictyostelium discoideum* (17); this organism also makes use of pleiotropic regulation by a differentiation factor to prevent cheating (18). We believe that an understanding of the selective pressures for maintenance of quorum sensing–controlled cooperation might lead to an ability to manage bacterial populations in a natural setting. We do not understand what forces might have selected quorum control of private goods together with public goods. Constraint on cheating is a consequence of this co-regulation but the co-regulation did not necessarily evolve for this purpose. Finally, social cheating will hamper the use of synthetic quorum-sensing circuits for production of secreted enzymes in biotechnological applications. Our results suggest one way in which synthetic systems could be engineered to suppress such phenomena.

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Acknowledgments: This work was supported by U.S. Public Health Service grants GM-59026 and P30 DK 89507 to E.P.G. A.A.D. is supported by a Cystic Fibrosis Foundation Leroy Matthews Fellowship. Data are deposited in the Dryad Repository: <http://dx.doi.org/10.5061/DRYAD.1225V>.

Supplementary Materials

www.sciencemag.org/cgi/content/full/338/6104/264/DC1
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11 July 2012; accepted 24 August 2012
10.1126/science.1227289

Quantifying the Impact of Human Mobility on Malaria

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Human movements contribute to the transmission of malaria on spatial scales that exceed the limits of mosquito dispersal. Identifying the sources and sinks of imported infections due to human travel and locating high-risk sites of parasite importation could greatly improve malaria control programs. Here, we use spatially explicit mobile phone data and malaria prevalence information from Kenya to identify the dynamics of human carriers that drive parasite importation between regions. Our analysis identifies importation routes that contribute to malaria epidemiology on regional spatial scales.

Local “hot spots” of malaria prevalence, resulting from complex interactions between the malaria parasite *Plasmodium falciparum* and its human and mosquito hosts, provide specific targets for the strategic deployment of malaria interventions (1–4). Movements of infected humans can increase the dispersal of parasites beyond what would be possible for mosquitoes alone (5, 6), and national malaria control programs must ac-

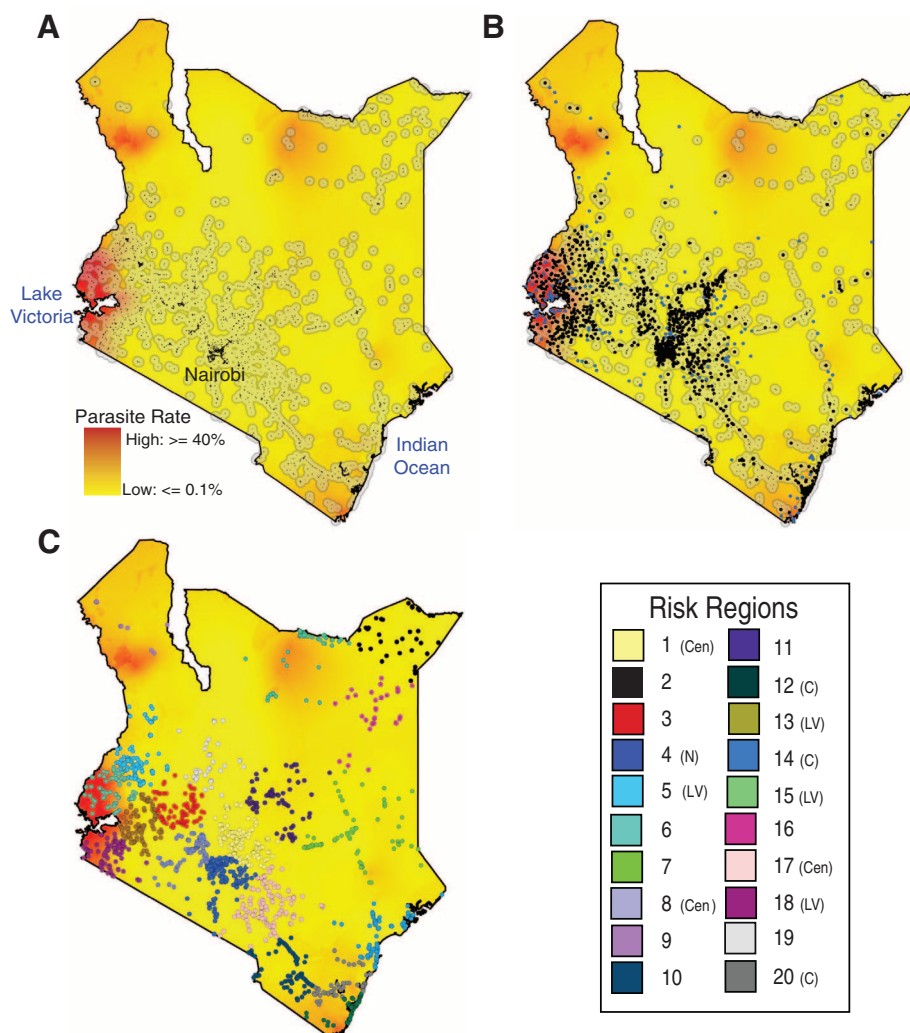
count for this human travel-mediated spread of parasites because frequent introduction of imported parasites could undermine local control or elimination strategies (5, 7–9). Mapping the routes of parasite dispersal by human carriers will allow for additional targeted control by identifying both the regions where imported infections originate and where they may contribute substantially to transmission. International migrants can contrib-

ute to continental parasite dispersal across Africa, and census surveys have provided insights into these routes of importation (6). The vast majority of travelers that will affect malaria parasite dispersal are those moving within a country between regions of variable malaria receptivity on a daily or weekly basis, however.

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Fig. 1. The distribution of settlements, cell towers, and malaria risk in Kenya. **(A)** Malaria prevalence in Kenya in 2009 (from $PfPR_{2-10} < 0.1\%$ in yellow to $PfPR_{2-10} > 40\%$ in red) and the locations of settlements used in the analysis (settlement centers are shown in black, and mapped with a 10-km extent around the perimeter of the settlement in gray). **(B)** The location of mobile phone towers (black or blue dots) and the extended settlement boundaries. Towers that fall within a settlement are shown in black, and those excluded from the analysis are shown in blue. **(C)** Regions used for visual mapping of transmission routes. Each settlement was allocated to 1 of 20 regions by a clustering algorithm (14) on the basis of homogeneous malaria risk and geography, as shown. Regions near Lake Victoria (LV), in Nairobi (Nairobi), the central areas (Cen), and along the coast (C) are labeled accordingly.



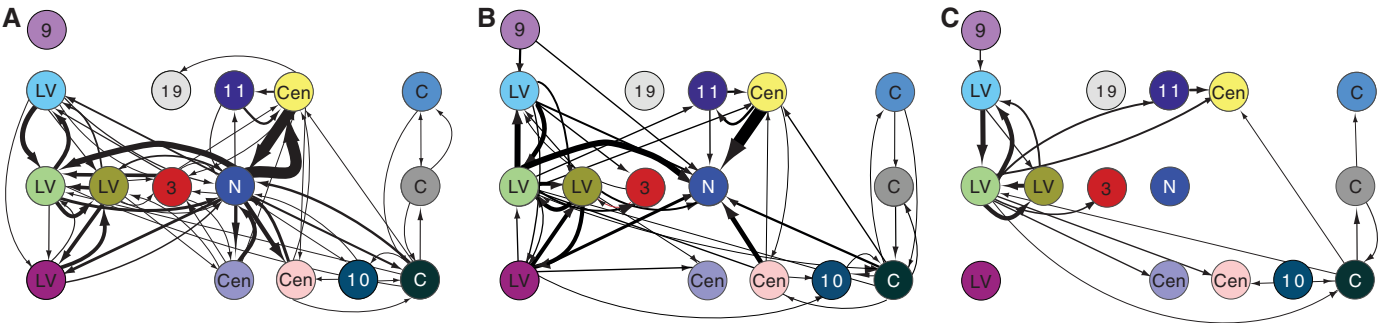


Fig. 2. Travel networks of people and parasites between settlements and regions. **(A)** Average monthly travel between regions (nodes), with edges weighted by volume of traffic. For clarity, the top 50% of routes are shown with arrows indicating the direction of movement (humans or parasites) from a

primary settlement to a visited settlement. **(B)** Average monthly parasite importation by returning residents, by region. **(C)** Average monthly parasite importation by visitors, where importation is only considered if the destination is receptive to onward transmission (14). Nodes are colored and labeled as described in Fig. 1.

Here, we use mobile phone data to analyze the regional travel patterns of nearly 15 million individuals over the course of a year in Kenya. We combine these data with a simple transmission model of malaria based on highly spatially resolved malaria infection prevalence data to map routes of parasite dispersal. Previous small-scale studies have used mobile phones to estimate importation rates of malaria parasites by residents of Zanzibar after journeys to mainland Tanzania, but these data lacked resolution on the infection risk at their journey destinations, as well as information about infected visitors to the island (8–10). Here, we identify networks of parasite movements across Kenya and pinpoint both “source” and “sink” regions.

We estimated the daily locations of 14,816,521 Kenyan mobile phone subscribers between June 2008 and June 2009, mapping every call or text made by each individual to one of 11,920 cell towers located within the boundaries of 692 settlements (Fig. 1, A and B) that were defined by satellite imagery as previously described (11–14). Each individual was assigned to a primary settlement where they spent the majority of their time over the course of the year, and the destination and duration of each journey made out of the primary settlement were calculated (fig. S1). We used a malaria prevalence map from 2009 (15) with a 1-km² resolution to assign each settlement a malaria endemicity class ranging from 1 (<0.1% prevalence of *Plasmodium falciparum* infection in 2- to 10-year-olds, PfPR₂₋₁₀) to 7 (≥40% PfPR₂₋₁₀), and these estimates were used to infer (i) a resident’s probability of being infected and (ii) the daily (nightly) probability that visitors to the settlement will become infected. Data on the seasonality of infection risk was not available, so these estimates likely represent an upper bound (14). Settlements were grouped into risk regions via a clustering algorithm to define geographically contiguous groups with the same malaria endemicity (Fig. 1C) (14).

The travel network (Fig. 2A and fig. S2A) is dominated by the Kenyan capital Nairobi, which forms a hub for human movements to and from all regions of the country. Although the highest

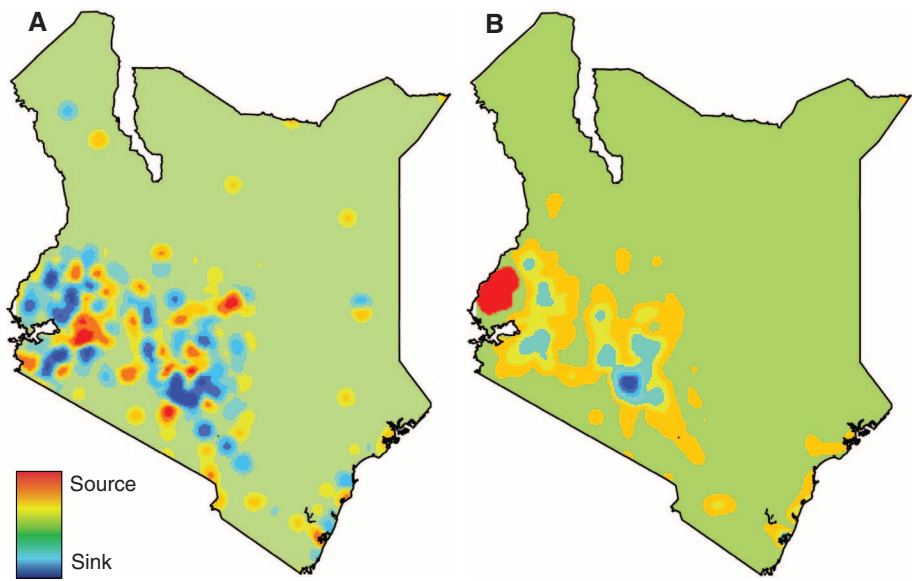


Fig. 3. Sources and sinks of people and parasites. Kernel density maps showing ranked sources (red) and sinks (blue) of human travel and total parasite movement in Kenya, where each settlement was designated as a relative source or sink based on yearly estimates. **(A)** Travel sources and sinks. **(B)** Parasite sources and sinks.

volume of travel occurs between Nairobi and the central regions of the country, substantial movement also occurs between the central region and Lake Victoria (for values, see tables S1 and S2).

There are two sources of importation of parasites. First, individuals visiting endemic areas may become infected during their stay, depending on the malaria endemicity of the destination, carrying parasites back to their primary settlement (14). We term these individuals “returning residents” and they are equivalent to “passive acquirers” of infections (1). Parasite networks resulting from travel by returning residents are shown in Fig. 2B (see fig. S2B and tables S3 and S4). Second, infected individuals can carry parasites with them when they visit other settlements, which potentially contribute to onward infections if the destination is receptive to transmission (14). The network of parasite movement by “visitors” is illustrated in Fig. 2C, and these individuals are equivalent to “active trans-

mitters” in previous frameworks (1) (fig. S2C and tables S5 and S6). For this analysis, we assume that receptivity to transmission is reflected by the prevalence of infection, although this simplification does not account for current control measures, which we discuss below. The structures of these networks were remarkably stable over the course of the year (see figs. S3 to S5), so, although seasonal changes in transmission might cause our estimates of parasite movement to be generally high, the routes and relative volumes will remain unaffected.

Parasite movement networks represent only a subset of the human mobility network underlying them, because of the spatial heterogeneity in malaria risk across the country. The human travel network is denser than the parasite networks, as expected, with more edges and a higher mean degree per settlement, as well as greater connectivity (see table S7). Returning residents contribute to some movements of parasites between

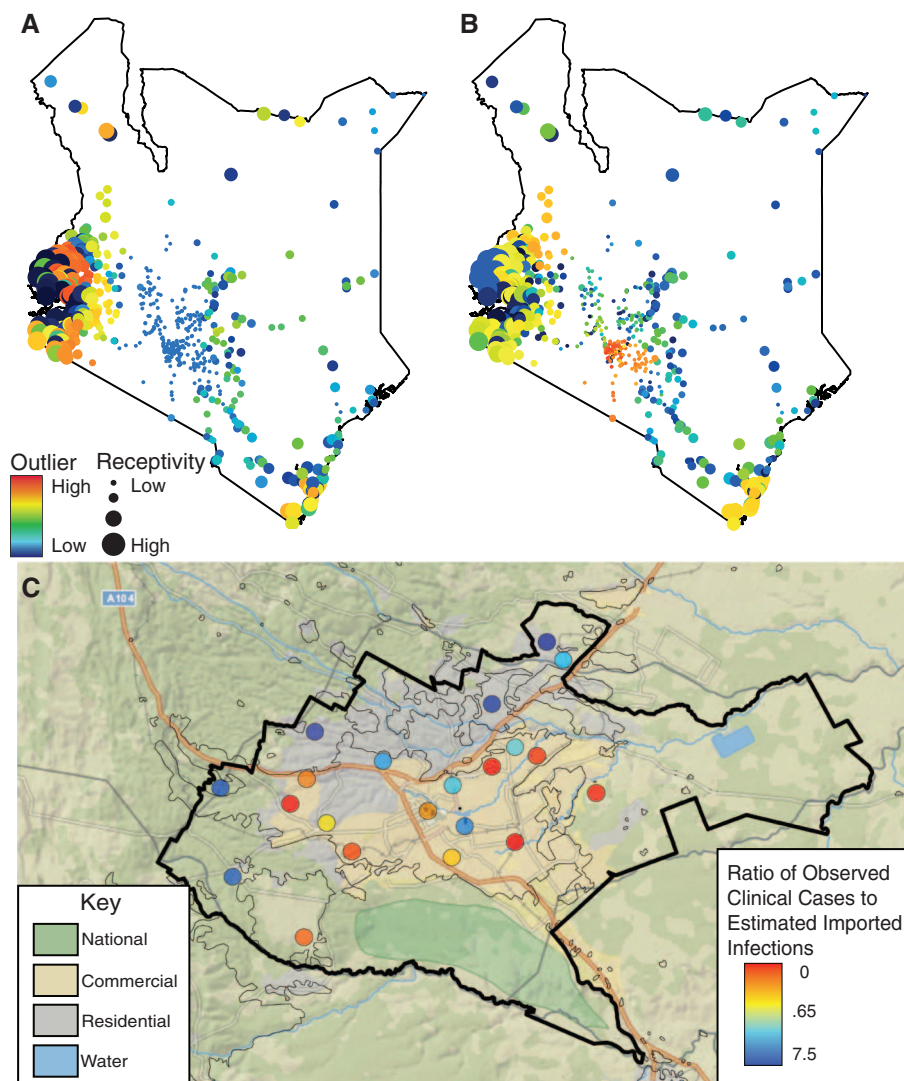


Fig. 4. Local analysis of source-sink anomalies. **(A)** Source outliers and **(B)** sink outliers. Settlements are colored by their outlier rank (from low values in blue to high values in red) and sized according to R_c , an indicator of receptivity. **(C)** The ratio of estimated localized importation to malaria cases at clinics around Nairobi. A topographic map of the city was from National Geographic, and the Economic and Social Research Institute's geographic information system highlights the national park, commercial, and residential areas.

regions within the Lake Victoria and coastal areas (Fig. 2B), but Nairobi imports the largest fraction of infections in this way, with infected residents returning after journeys to the coast, Lake Victoria, and low-endemicity regions in central Kenya. Visitors contribute to transmission anywhere that is receptive to transmission (14) (Fig. 2C), but may have less impact in the capital, for example, where vectors are scarce. Hence, the visitor network is dominated by importation around Lake Victoria and shows relatively low importation rates between the lake and the coast, the two main foci of transmission. Visitors carrying parasites within regions are therefore likely to be a much more important consideration for control programs than interregional visitors, which suggests that the Lake Victoria and coastal regions may be considered as weakly connected, but relatively

independent, entities for the purposes of malaria elimination.

To examine directional and net movements of people and parasites between settlements, we analyzed asymmetries between “source” and “sink” settlements. Here, we rank settlements based on their contribution as net emitters (sources) and net receivers (sinks) of people and parasites (human travel in Fig. 3A, parasite movement in Fig. 3B) (14). The difference between each settlement's source and sink rank distinguishes those that are primarily sources of people or parasites versus those that are primarily sinks. Sources and sinks of human travel are almost entirely overlapping and reflect patterns of population density and regular travel (Fig. 3A). In contrast, the parasite routes show directional movement between source settlements in the Lake Victoria region

and parasite sinks on the periphery of this focus of transmission and in the Nairobi area (Fig. 3B) (14). The capital city and its surroundings are thus a major destination for both humans and parasites, but most of the parasite importation that can contribute to onward transmission occurs on the periphery of the highly endemic Lake Victoria region. Therefore, even though malaria prevalence is low in these regions and so amenable to control, elimination programs must account for imported infections to be successful.

The high spatial resolution of our mobility data allowed us to pinpoint particular settlements that are expected to receive or transmit an unexpectedly high volume of parasites compared with surrounding regions. The result of an analysis of outlying settlements identified by means of an anomaly detection algorithm is shown (Fig. 4, A and B) (14). Here, the size of the circle represents R_c , the basic reproductive number of the parasite under control (16). This measure provides insights into how important outliers are likely to be for transmission, because importation can only contribute to transmission if local conditions and vector populations allow it. Combining local estimates of importation with information about locally heterogeneous transmission—including vector behavior, ecology, and population distributions on a fine scale—will play an important role in future regional elimination efforts. Again, the settlements on the edge of Lake Victoria are major sources of parasites, and the neighboring settlements farther inland are most vulnerable to importation. Returning residents played an important role in importing parasites to major parasite sinks, with residents from the top 10% of outlying settlements taking, on average, 29 trips during the year, compared with 20 trips by individuals from the remaining 90% of settlements (medians 10.4 versus 7.6, respectively, Mann-Whitney U test, $P < 0.0001$). These sinks also received substantial numbers of visitors from higher malaria endemicity settlements (24% of visitors) compared with settlements that were not considered sinks (12% of visitors). In contrast, individuals from the top 10% of major parasite source settlements did not travel more frequently, but 62% of journeys made were to settlements with lower malaria endemicity compared with 0.08% of journeys made from the remaining 90% of settlements ($P < 0.0001$) (tables S8 and S9).

In Nairobi, the density of cell towers enabled further localization of these estimates and a comparison with cross-sectional clinical surveys of malaria incidence carried out in 2010 (17). Frequent malaria epidemics occurred in the capital at the beginning of the 20th century but declined markedly after substantial control efforts, rapid population growth, and urbanization. The current potential for local transmission within the city is controversial, with studies showing substantial infection prevalence and ongoing treatment of presumed clinical cases, despite the scarcity of suitable mosquito vectors (18–20). The ratio of monthly clinical cases to our predicted monthly imported

cases from mobile phone data at the location of each hospital survey is shown (Fig. 4C) (14) (table S10 and fig. S6). Areas in the highly urbanized center of the city, where transmission is unlikely, show a very large ratio of estimated imported-to-clinical cases. In contrast, hospitals on the periphery of the city have a higher ratio of clinical cases to estimates from the mobile phone data. The patterns suggest some local transmission may be occurring in these residential and less developed areas and could increase if migration into the areas surrounding the city is not accompanied by improved public health infrastructure and surveillance programs. Poor malaria monitoring in clinics around the city is currently hindering the accurate assessment of malaria transmission (17). Although caution must be exercised in the interpretation of comparisons between clinical and mobile phone estimates, this approach provides a starting point for the identification of transmission foci in low-risk urban settings and the local implementation of surveillance programs.

There are limitations to this approach (10), because we can only measure mobility among phone owners in areas where there are cell towers (21) (see supplementary materials for discussion), we cannot capture cross-border migration, and our importation calculations are constrained by the available, nonseasonal malaria prevalence estimates. Nevertheless, this analysis has made it possible to assess the degree of connectivity among different regions of Kenya—the resulting estimates can be used to estimate costs for regional elimination strategies, identify “source” regions where reducing transmission would provide benefit to surrounding areas, evaluate patterns of importation and endemicity in low-intensity

areas such as Nairobi, and pinpoint likely importation hot spots. On an extremely local scale, driven primarily by vector biology and habitat and local variability in household structures, hot spots of transmission can be targeted by indoor residual spraying, vector habitat removal, insecticides, drug administration, and bed-net use. Control-program activities targeting the large volumes of human traffic between regions that we have identified here will be completely different from those that concentrate on local transmission hot spots, focusing on communicating risks to travelers to alter their behaviors, restricting travel patterns, and/or conducting routine surveillance in high-risk areas.

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Acknowledgments: A.W. was supported by the National Science Foundation Graduate Research Fellowship program (0750271). D.L.S. and A.J.T. acknowledge support from Bill & Melinda Gates Foundation grants (49446 and OPP1032350); National Institute of Allergy and Infectious Disease, NIH, grant (U19AI089674); and the Research and Policy for Infectious Disease Dynamics (RAPIDD) program of the Science and Technology Directorate, Department of Homeland Security; and the Fogarty International Center, NIH. A.M.N. is supported by the Wellcome Trust as an Intermediate Research Fellow (095127). R.W.S. is supported by the Wellcome Trust as Principal Research Fellow (079080). A.M.N. and R.W.S. also acknowledge programmatic support provided by the Wellcome Trust Major Overseas Programme grant to the Kenya Medical Research Institute–Wellcome Trust Programme (092654). C.O.B. was supported by the Models of Infectious Disease Agent Study program (cooperative agreement 1U54GM088558). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of General Medical Sciences or the NIH. Mobile phone data were provided by an anonymous service provider in Kenya and are not available for distribution.

Supplementary Materials

www.sciencemag.org/cgi/content/full/338/6104/267/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S6
Tables S1 to S10
References (22–28)

17 April 2012; accepted 29 August 2012
10.1126/science.1223467

Preference by Association: How Memory Mechanisms in the Hippocampus Bias Decisions

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Every day people make new choices between alternatives that they have never directly experienced. Yet, such decisions are often made rapidly and confidently. Here, we show that the hippocampus, traditionally known for its role in building long-term declarative memories, enables the spread of value across memories, thereby guiding decisions between new choice options. Using functional brain imaging in humans, we discovered that giving people monetary rewards led to activation of a preestablished network of memories, spreading the positive value of reward to nonrewarded items stored in memory. Later, people were biased to choose these nonrewarded items. This decision bias was predicted by activity in the hippocampus, reactivation of associated memories, and connectivity between memory and reward regions in the brain. These findings explain how choices among new alternatives emerge automatically from the associative mechanisms by which the brain builds memories. Further, our findings demonstrate a previously unknown role for the hippocampus in value-based decisions.

Decisions are sometimes guided by direct past experience: If a choice led to a good outcome in the past, people are likely

to make that same choice again. This process is known to depend on reward learning mechanisms in the striatum (1, 2). But frequently in

life, we have to decide between options we have never considered before. It has been suggested that such decisions could be guided by associative memory (3–5); however, surprisingly little is known about how this process happens.

We investigated the mechanism by which neural circuits for memory modulate value and guide decisions about new choice options. Our central hypothesis was that the hippocampus enables the positive value of reward to spread across associated memories, thereby increasing the value of items that were never rewarded. Specifically, we hypothesized that receiving reward can lead to two simultaneous and interactive processes: (i) the direct learning of stimulus-reward associations in the striatum and (ii) the spread of reward to associated items stored in memory via the hippocampus.

Our hypothesis is grounded in two essential features of how the hippocampus builds memories. First, the hippocampus encodes relationships

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between items and events that appear together, forming an associative link between them (6–8). Second, because of this associative link, when a person later encounters one item, the hippocampus can complete the pattern and automatically reactivate the neural representation of the other item (9–12), allowing the integration of old memories with new ones.

We reasoned that these features of memory formation in the hippocampus could provide a mechanism by which reward experiences can systematically change the value of items that were never rewarded. These items gain a positive value merely by association. If so, this mechanism predicts that later, when confronted with a decision, people will be biased to choose items that were never rewarded in the past (4, 13–15). By emphasizing the associative nature of processes in the hippocampus, regardless of awareness (7, 8, 16, 17), this mechanism further

predicts that the hippocampus might bias value even when associations are not explicitly remembered.

To test our prediction that reward will spread across associated memories, we used functional magnetic resonance imaging to measure brain responses during a learning and decision task designed to test how associative memory biases decisions between new choice options (Fig. 1) (13). First, we had participants ($n = 28$) build new associative memories by exposing them to regularities between pairs of neutral stimuli (denoted here as S_1 and S_2). These regularities were encoded incidentally while participants performed a cover task (Fig. 1A).

Next, we associated value with some items by using a classical conditioning paradigm in which half of the S_2 stimuli (S_2+) were now followed by a monetary reward (Fig. 1B). This procedure is known to enhance the value of the directly re-

warded items via well-described reward learning mechanisms in the striatum (2). Critically, we hypothesized that at the same time, associative memory processes in the hippocampus would activate the specific S_1 items associated with the rewarded S_2 items, resulting in a transfer of the reward to the S_1 items as well, through hippocampal-striatal connectivity (18, 19). This would increase the value of S_1 items that were linked to the rewarded S_2 items (S_1+), creating a bias toward choosing these items in the future, despite the fact that these items were never rewarded and were not even seen during reward learning.

To measure the effect of associative memory on value, in the final phase of our experiment, we asked participants to make a series of decisions in which they had to choose between two S_1 items, selecting the “luckier” one for potential winnings, awarded at the end of the experiment (Fig. 1C, top). In the absence of any spread of reward,

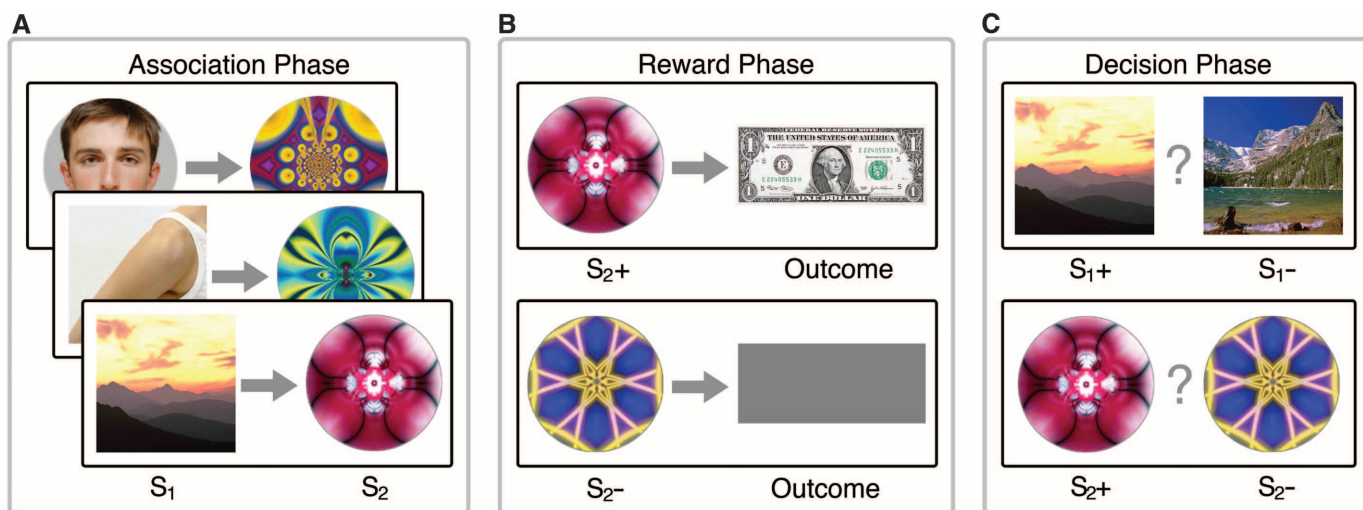
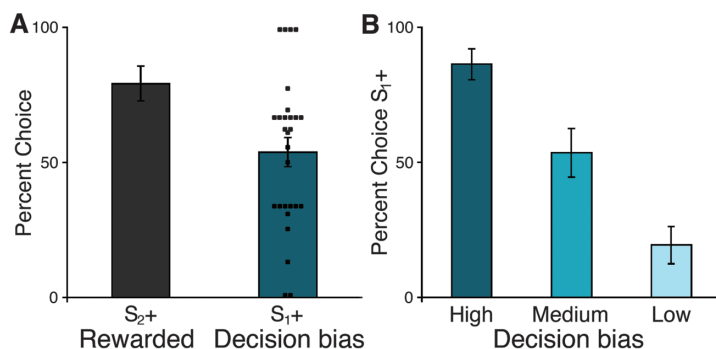


Fig. 1. The task consists of three phases: association learning, reward learning, and decision-making. (A) In the association phase, participants were exposed to a series of pairs of pictures (S_1 and S_2 stimuli) while performing a cover task to detect “target” upside-down pictures. S_1 stimuli were either face, scene, or body part pictures; S_2 stimuli were circle images. (B) In the reward phase, participants learned through classical conditioning that half of the S_2 stimuli were followed

by a monetary reward (S_2+), whereas the other S_2 stimuli were followed by a neutral outcome (no reward, S_2-). S_1 stimuli never appeared in this stage. (C) In the decision phase, participants were asked to decide between two stimuli (both S_1 or both S_2) for a possible monetary win. No feedback was provided, and all gains were awarded at the end of the experiment. Decision bias was operationalized as the tendency to choose S_1+ over S_1- stimuli in this phase.

Fig. 2. Decision bias varies within and across participants and is related to activation in the hippocampus during the reward phase. (A) Decision-phase preferences for S_2+ stimuli (dark gray) and S_1+ stimuli (average, blue; within-participant mean, black) are shown. Error bars represent $\pm$ SEM. (B) Within-participant decision bias variability. Each participant was exposed to three different types of stimuli, allowing for differential bias across associations. Individual mean decision bias [shown in (A)] was separated, ranked by level of bias, and averaged across participants. (C) Hippocampal activation within participants during the reward phase



(S_2 stimulus presentation and outcome) predicted subsequent levels of decision bias (for associated S_1 stimuli) [z score = 4.00 (26, -34, -12); $P < 0.05$, SVC; images thresholded at $P < 0.005$, uncorrected for display]. R, right.

participants should be equally likely to choose any of these nonrewarded items, and brain activity during the prior reward phase should be unrelated to these decisions. However, if reward spreads and biases decisions, then participants should be biased toward choosing those nonrewarded S_1 items that were previously associated with the S_2 rewarded items. Thus, we operationalized “decision bias” as the tendency to choose S_{1+} items over S_{1-} items, and we hypothesized that decision bias should be related to neural processes in the hippocampus during reward learning.

To test this hypothesis, we focused our analyses on brain activity during the reward phase and asked whether activity during this phase was related to later biases in decisions. We made three specific predictions about the neural mechanisms giving rise to the spread of value to new choice options: (i) If associative memory processes underlie shifts in value, then biased decisions should be predicted by the magnitude of activation in the hippocampus during reward learning. (ii) If reactivation of associations is the mechanism by which value spreads, then during reward learning there should be evidence of neural reactivation in visual areas that represent the associated S_1 items. (iii) If decision bias stems from interactions between associative memory processes in the hippocampus and reward learning processes in the striatum, then decision bias should be related to functional connectivity between these two regions.

Behaviorally, participants tended to choose the S_{2+} items over the S_{2-} items in the decision phase, indicating successful reward learning. Interestingly, decision bias in favor of S_{1+} items varied markedly both within and across individuals: Most participants displayed a bias in favor of S_{1+} items, but some did not (Fig. 2A). Within individuals, this measure of bias was strong for some associations, but weaker for others. This variability in behavior

allowed us to ask: What are the neural mechanisms that support decision bias?

To test our first prediction that decision bias is related to hippocampal activity, we used a general linear model to compare blood oxygen level–dependent (BOLD) activity during the reward phase between items that led to later behavioral decision bias versus those that did not, i.e., $S_{1+} > S_{1-}$, within individuals (Fig. 2B). Activation in the posterior hippocampus during reward learning was greater for items that led to more decision bias (Fig. 2C). A similar pattern was found across individuals: Activation in the hippocampus correlated with the proportion of biases in subsequent decisions [Montreal Neurological Institute (MNI) space coordinates (R, A, S) 30, –6, –20; $P < 0.05$ small-volume corrected (SVC) for familywise error]. As would be expected if decision bias is driven by the spread of value via associative memory processes, neural predictors of bias were selective to the reward phase. Parallel analyses of BOLD activity during the association and decision phases showed that no areas of the brain within or across participants had activation correlated with decision bias.

To investigate whether hippocampal activity was related to explicit memory for the S_1 – S_2 associations, after scanning we tested participants’ ability to correctly pair S_1 and S_2 items and asked about their choice strategy and awareness of task structure (20). Reflecting the automatic nature of the underlying associative memory processes (13, 16), we found no evidence for explicit memory of the associations. Moreover, there was no relation between measures of explicit S_1 – S_2 memory and either decision bias or hippocampal activity (fig. S3) (20). Although it is difficult to conclusively determine implicitness of cue pairings (21), these findings suggest that the role of the hippocampus in decision bias does not seem to be driven by explicit or strategic effects.

To test our second prediction that decision bias is supported by reactivation of associations, we exploited the fact that the different categories of S_1 stimuli (faces, scenes, and body parts) elicit activation in distinct areas of the visual cortex (22). In our design, these category-specific S_1 items were associated with S_2 items in the association phase; in the reward phase, however, only S_2 items were presented. Thus, during reward learning, any selective activation in these visual cortical areas probably reflects associative reactivation, in memory, of these items. This allowed us to test whether biases in decisions, as measured behaviorally, were predicted by differential activation in category-specific areas during the reward phase.

We analyzed associative reactivation during the reward phase using activation masks defined by participants’ responses to S_1 visual stimuli during the association phase (Fig. 3A) (20). These participant-specific masks were then applied to reward-phase responses evoked by S_2 stimuli, relative to the alternative categories, resulting in a measure of reactivation for each participant for each association. We compared reactivation for associations that led to high versus low decision bias.

We found that reactivation in visual regions during reward learning related to later biases in decisions (Fig. 3), such that across all categories there was greater neural reactivation for high- versus low-bias decisions. Reactivation was significant in the first half of the reward phase. Reflecting the continual updating of memory representations, it was present but weaker over the full reward phase, as additional learning about the S_2 items took place. Importantly, reactivation was selective to the associated category-specific regions and was not general to all visual regions of interest (20).

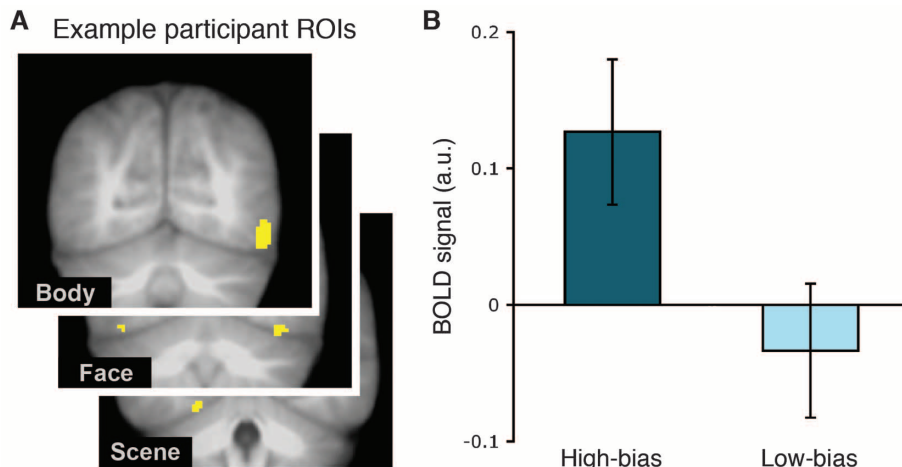


Fig. 3. Reactivation of category-specific visual areas during the first half of the reward phase is related to subsequent decision bias. (A) Example participant region of interest masks (derived from the association phase) for body, face, and scene S_1 stimuli. Masks were applied to S_2 presentations during the reward phase. (B) S_2 presentation elicits activation in visual regions responsive to associated S_1 stimuli when participants later exhibit decision bias [$t(22) = 2.29$, $P < 0.05$]. Error bars indicate $\pm$ SEM; a.u., arbitrary units.

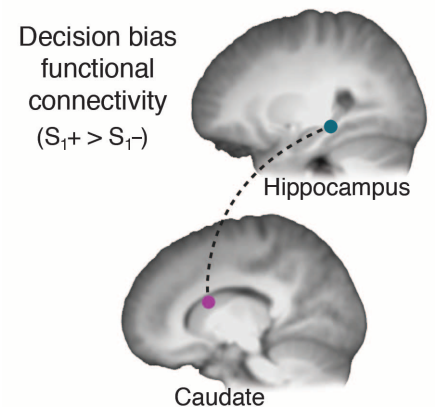


Fig. 4. Decision bias was related to functional connectivity between the hippocampus and the striatum during the reward phase. A PPI analysis revealed correlated activity between the hippocampus and the striatum during trials that led to high versus low decision bias. The same region in the striatum was also found to correlate with reward learning. This finding indicates that the hippocampus and the striatum may form a functional circuit to support shifts in value.

Finally, we tested our third prediction that decision bias is related to functional interactions between the hippocampus and the striatum during the reward phase. Specifically, we reasoned that an interaction between associative reactivation of S_1 stimuli via the hippocampus and reward learning in the striatum could provide a link between S_1 stimuli and reward.

We investigated functional connectivity during the reward phase by conducting a psychophysiological interaction (PPI) analysis using the right hippocampus as a seed and decision bias (high versus low) as the behavioral (psychological) modulator. We found a functional relation between the hippocampus and the striatum; this relation was significantly greater for high-bias stimuli [z score = 3.23 (6, 6, 12); $P < 0.05$ SVC] (Fig. 4) (20).

We then investigated whether the correlation between the hippocampus and the striatum for high-bias decisions is mediated by reactivation in visual cortical regions. To test this, we extracted trial-by-trial measures of evoked responses to high-bias stimuli and performed a formal mediation analysis on the path between the hippocampus and the striatum via the potential visual cortex mediator (20). Consistent with the PPI result, we found that high decision bias was related to correlated BOLD activity in the hippocampus and striatum (0.214 ± 0.054 , $P < 0.001$). Additionally, we found a trend for a mediating effect of visual reactivation on this relationship (0.010 ± 0.006 , $P < 0.08$) (20).

To test for the specificity of these connectivity results, we conducted a series of control analyses (20). In a PPI analysis of the association phase, we found no areas with differential connectivity that related to decision bias, and in the reward phase, we found no significant activation outside of the striatum. Furthermore, in the mediation analysis, a control analysis of regions that were activated by the reward phase did not show any connectivity with regions of interest for high-bias stimuli (20). Together, these results indicate that decision bias depends not only on hippocampal activation during reward learning, but additionally on functional connectivity between the hippocampus and the striatum, putatively mediated by reactivation in visual cortical regions.

These results indicate that reward can spread to bias the value of options that were themselves never directly rewarded (13, 15). This finding provides insight into how people are biased by past experience to make new decisions between options that were never previously rewarded: Networks of associations in memory, formed across many different experiences, can result in the spread of value across associations.

The idea that memory and decision-making are intertwined has deep roots in behavioral theories of both memory (3, 7, 18) and decision-making (23–26), yet there has been very little evidence for an underlying mechanism. Understanding the mechanism by which value spreads among related memories in the brain may help to explain why people sometimes develop seemingly ungrounded preferences for or against particular things, places, or people. Although we highlight how transfer of past experience can guide behavior in a changing environment, this same mechanism may also lead to seemingly irrational choices, consistent with social and cognitive theories regarding the role of associative memory in decision-making heuristics (23–27).

The finding that the hippocampus supports the spread of value provides several new insights into the neural bases of both memory and decision-making. First, our findings extend the role of the hippocampus beyond memory per se, demonstrating that the hippocampus contributes directly to value assignment and decision-making.

Second, although in humans the hippocampus is traditionally associated with explicit declarative memory (7), our results indicate that transfer of value by the hippocampus is not driven by conscious awareness. Thus, our results suggest that the hippocampus contributes to an automatic assessment of value, perhaps performing a function similar to Bayesian inference about value (28).

Finally, though it is known that memory can support decisions by retrieving relevant information at the time of decision-making (29, 30), our results demonstrate an alternative mechanism whereby the hippocampus dynamically modulates value representations during learning itself (5, 31, 32). This mechanism allows value to spread and bias decisions without effortful retrieval at the time of decision.

Understanding how associative memory biases decisions provides insight into critical open questions in decision-making research. Although reward learning models have been successfully applied to many aspects of behavior, these models cannot account for the full diversity of animal and human decision-making (2, 33, 34). The uncovering of a neural mechanism by which associative memory biases decision-making sheds light on how value generalizes across experience, with implications for both adaptive behaviors and maladaptive behaviors such as addiction.

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Acknowledgments: Funding was provided by a NSF Career Development Award and National Institutes of Drug Abuse grant R03-DA026957 to D.S. We thank E. K. Braun, L. Davachi, R. Hassin, and E. Weber for helpful comments on an earlier draft of the manuscript; L. Atlas and T. Wager for guidance with the mediation analyses; and N. Daw and R. Hassin for insightful conversations.

Supplementary Materials

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Materials and Methods
Supplementary Text
Figs. S1 to S5
References (35–54)

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Academia Sinica, the most prominent academic institution in Taiwan is comprised of world-class institutes. The infrastructure for research is excellent, and Research Fellows are afforded well-equipped laboratories and access to excellent research funding opportunities. The ABRC of Academia Sinica pursues basic and applied research in agricultural biotechnology. For more information of ABRC and Academia Sinica, please visit our websites at <http://abrc.sinica.edu.tw/> and http://www.sinica.edu.tw/main_e.shtml, respectively.

Qualifications: Ph.D. in plant science and plant biotechnology or related field with an outstanding record of research achievement. We are particularly interested in applicants who are seeking a highly collaborative research environment.

Applicants should submit the following materials online, at <http://abrc.sinica.edu.tw/jobs/>: (a) Cover letter; (b) Curriculum vitae, including publications; (c) A summary of research accomplishments; (d) Clearly focused description of future research plans; (e) PDF copies of major publications; (f) Names and contact information for three referees. Candidates should arrange three letters of recommendation to be submitted by e-mail to: abrc@gate.sinica.edu.tw or sent by regular mail to: **Faculty Search Committee, Agricultural Biotechnology Research Center, Academia Sinica, No. 128, Academia Rd. Sec. 2, Nankang, Taipei 11529, Taiwan.** Review of candidates will begin on **December 1, 2012** and continue until the positions are filled.



UNC
LINEBERGER

Basic/Translational Cancer Research Faculty

The UNC Lineberger Comprehensive Cancer Center, in collaboration with departments in the School of Medicine and across the entire University of North Carolina Chapel Hill, seeks outstanding candidates for faculty positions at all levels and at all ranks in basic and translational cancer research with a special interest in a senior cancer researcher. This broadbased recruitment seeks outstanding scientists in a number of areas, including but not limited to: animal models, signal transduction, cancer genetics, bioinformatics, virology, drug development and target validation, epigenetics and gene expression, DNA damage and repair, cancer therapy, cancer immunology, inflammation and cancer, and stem cells. Applicants should have a strong record of recent accomplishments as a post-doctoral fellow or sustained productivity as an established faculty member. Appointment and rank in an academic department will be determined by the applicant's qualifications. Applications will be reviewed beginning **December 1, 2012** and until the positions are filled.

Educational Requirements: Doctoral Degree

Qualifications and Experience: Doctoral Degree

Please apply on line at <http://unc.peopleadmin.com/postings/8803> and provide curriculum vitae, a description of research plans and contact information of four references.

*University of North Carolina at Chapel Hill is an
Equal Opportunity/ADA Employer.*

ONE TEST

And a family is reunited

Angela was 21 days old when she was kidnapped in broad daylight. Her mother, Brenda, was on her way to the doctor when two women assaulted her, snatched Angela from her arms, and took off in a car in Guatemala City.

Two months passed before Angela was found. If it were not for a DNA test—a test created by Life Technologies—there would have been no proof Angela belonged to Brenda.

The test result revealed a 99.9% positive DNA match. Finally, Angela got to go home.

Shaping Discovery.
Improving Life.



Angela and Brenda Corado



Join in to stand out. Go to lifetechnologies.com/careers

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life
technologies™



THE HONG KONG UNIVERSITY OF SCIENCE AND TECHNOLOGY

Division of Life Science Faculty Positions

The Division of Life Science at The Hong Kong University of Science and Technology seeks applicants for multiple tenure-track positions at the rank of Assistant or Associate Professor. Although applications from outstanding individuals in any area of the life sciences will be considered, the Division is most interested in the areas of systems biology, immunology, aging and stem cell biology. The Division is also seeking additional applicants to expand its marine and environmental sciences. We encourage applications from individuals who use contemporary model systems and/or modern approaches to these areas. In all of these disciplines we are looking for candidates whose work is grounded in basic biology yet maintains a clear connection to the problems of human health and well-being. Successful applicants should have a doctoral degree as well as postdoctoral experience. They will be expected to establish an independent, internationally recognized research program and to contribute to the undergraduate and graduate teaching missions of the Division.

The Division of Life Science (<http://life-sci.ust.hk/>) was established in 2010 through the integration of the Departments of Biochemistry and Biology. The 29 current faculty members are experts in a broad range of areas including macromolecular structure and function, cell biology and signalling, cancer biology, developmental biology, molecular and cellular neuroscience, marine and environmental science, and biotechnology and medicinal biochemistry. The Division is located in the vibrant international atmosphere of The Hong Kong University of Science and Technology, located in a quiet, picturesque sea-side setting, just 40 minutes from downtown Hong Kong. Teaching and research are carried out in an outstanding intellectual environment that is rich in technical resources. The medium of instruction is English.

Starting salary will be commensurate with qualifications and experience. Fringe benefits including medical and dental benefits and annual leave will be provided. Housing benefits will also be provided where applicable. The Division is committed to diversity in its ranks and strongly supports equal opportunity employment.

Application Procedure

Applications should include a curriculum vitae, a short statement of research interests and the names and addresses of three individuals who can serve as references for the candidate. These materials should be sent to **Prof. Yung Hou Wong, Chair of Life Science Search and Appointments Committee, Division of Life Science, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong**. Electronic submissions are strongly encouraged (email: lfsearch@ust.hk). Review of applications will start in October 2012 and will continue until early 2013.

(Information provided by applicants will be used for recruitment and other employment-related purposes.)



The University of Georgia

TWO TENURE TRACK FACULTY POSITIONS IN MOLECULAR MEDICINE

The University of Georgia announces openings for two tenure track faculty positions in its new Center for Molecular Medicine (CMM). Development of the CMM complements UGA's recent thrust into human health which is marked by the opening of a new health sciences campus that includes a medical school campus operated in partnership with Georgia Health Sciences University as well as the rapidly expanding College of Public Health. The CMM, which is dedicated to better understanding the molecular and cellular basis of human disease as well as developing new disease diagnostics, therapeutics and vaccines, is currently seeking applications from talented and accomplished investigators at the Assistant or Associate Professor level. Candidates utilizing cutting-edge molecular approaches in studies of human disease etiology, tissue regeneration and repair, cancer and stem cell biology, or the application of glycoscience to medicine are especially encouraged to apply.

Applicants for the Assistant level position must have a Ph.D. degree (or equivalent), at least two years relevant postdoctoral research experience, an outstanding publication record and a well-developed research plan. Applicants for the Associate position must have a Ph.D. with a demonstrated track record of outstanding independent research productivity over a span of at least 5 years. The successful candidates will be expected to establish a vibrant, extramurally funded research program. An attractive start-up package with highly competitive conditions of employment will be offered. The University of Georgia (www.uga.edu) is a land grant/sea institution located ninety miles northeast of Atlanta (www.visitathensga.com), offers an outstanding quality of life in the Athens region (www.georgia.gov), and is an EEO/AA institution.

To apply, submit a cover letter, CV, and a description of research interests to CMMSEARCH@ccrc.uga.edu electronically (indicate 'CMM Open Search' in the subject line). Applicants should arrange to have at least three letters of reference sent to: **Open Search Committee Chair, c/o Ms. Karen Howard, Center for Molecular Medicine, University of Georgia, CCRC Building, 315 Riverbend Road, Athens, GA 30602-4712**. Electronic letters of recommendation on letterhead are acceptable. Complete applications received by **January 25th, 2013** are assured full consideration.



UNIVERSITY OF
LIVERPOOL



FIAT

School of Physical Sciences
Department of Chemistry

Postdoctoral Research Associate – KCMC Project Scientist

£31,020 - £35,939 pa

Applications are invited for a Project Scientist to work in the Knowledge Centre for Materials Chemistry (KCMC). The KCMC is an activity uniting Chemistry Innovation Ltd, Science & Technology Facilities Council and the Universities of Liverpool, Manchester and Bolton to focus on delivering industry-led projects.

You should have an excellent research track record with experience in discovery and characterisation of new inorganic materials, with a PhD in chemistry, physics or materials science; excellent communication and interpersonal skills with the ability to interact with individuals at all levels within the academic and business communities. The post is available for 2 years.

Job Ref: R-580799/S

Closing Date: 2 November 2012

For full details, or to request an application pack, visit www.liv.ac.uk/working/job_vacancies/ or e-mail jobs@liv.ac.uk Please quote job ref in all enquiries.

COMMITTED TO DIVERSITY AND
EQUALITY OF OPPORTUNITY



Stonewall
DIVERSITY CHAMPION



Penn
UNIVERSITY OF PENNSYLVANIA

EARTH AND ENVIRONMENTAL SCIENCE Professorship in Earth and Environmental Science

The Department of Earth and Environmental Science at the University of Pennsylvania invites applications for a tenured professorship at the Associate or Full Professor level. We seek an individual with research and teaching interests that complement and broaden our existing strengths in both Earth and Environmental Science. The successful candidate is expected to have developed an internationally recognized, externally funded, multi-disciplinary research program and will be required to actively participate in our core undergraduate and graduate teaching and in the administration of the Department. Individuals who can further increase interactions with other departments within the School of Arts and Sciences are strongly encouraged to apply. Further information about the Department may be sought at www.sas.upenn.edu/earth/.

Applicants apply online at facultysearches.provost.upenn.edu/applicants/Central?quickFind=51104 with a cover letter, CV, statements of research and teaching interests, and 3 publications. The Search Committee will begin to evaluate applications on **November 15, 2012** and will continue until the position is filled.

The University of Pennsylvania is an Affirmative Action/Equal Opportunity Employer and is strongly committed to establishing a diverse faculty: <http://www.upenn.edu/almanac/volumes/v58/n02/diversityplan.html>

UT SOUTHWESTERN MEDICAL CENTER AT DALLAS

ENDOWED SCHOLARS PROGRAM IN MEDICAL SCIENCE

Unique and highly competitive, **THE ENDOWED SCHOLARS PROGRAM IN MEDICAL SCIENCE** is designed to launch the next generation's scientific leaders on their biomedical research careers by providing seed money, research space and startup support for groundbreaking projects. The Endowed Scholars Program gives early-career investigators the chance to take risks, supported by the mentoring of experienced and highly distinguished established researchers at **The University of Texas Southwestern Medical Center at Dallas**.

As one of the foremost research institutions in the world, UT Southwestern, with four Nobel Prizes awarded to its faculty since 1985 and 18 members of the National Academy of Sciences, is poised to lead the way in a new era of scientific discovery in the 21st century. We conduct more than 3,500 research projects annually totaling more than \$400 million. Endowed Scholars have access to exceptional core facilities, which include DNA microarray services; electron microscopy; live-cell imaging; mouse gene knockouts, transgenesis and metabolic phenotyping; high-throughput chemical screening; and structural biology. UT Southwestern also is home to one of the nation's first 7-Tesla magnetic resonance imaging devices for human studies. UT Southwestern has a vibrant graduate school and nearly 4,400 medical, graduate and health professions students, residents and postdoctoral fellows.

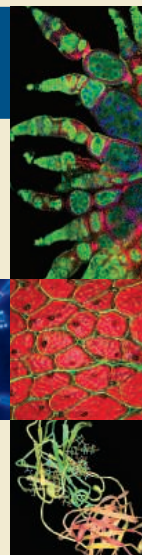
The Endowed Scholars Program, which is fully funded from private endowment, provides more than \$1,000,000 over four years to support the independent research activities, salary and benefits of each scholar. Up to five new scholars are selected each year from top universities, institutions and laboratories around the world. Each scholar is appointed as a tenure-track assistant professor in a UT Southwestern academic department or research center. Positions in both basic science and clinical departments are available.

Potential scholars submit nomination materials to a chair or director of one of UT Southwestern's basic or clinical academic departments or research centers. Those chairs or directors then forward finalists' materials for consideration to the medical center's Endowed Scholars Committee.

For detailed information about nomination materials and currently available positions, please visit our Web page: utsouthwestern.edu/utsw/home/scholars

*Applications from women and underrepresented minority candidates are strongly encouraged.
UT Southwestern is an equal opportunity institution.*

UT SOUTHWESTERN
MEDICAL CENTER



THE UNIVERSITY OF TEXAS

**MD Anderson
Cancer Center**

Making Cancer History®

A search for a new Chair of the Department of Biochemistry and Molecular Biology at The University of Texas MD Anderson Cancer Center is underway. We are seeking a candidate of international stature (Ph.D., M.D., or M.D./Ph.D.) interested in leading a world-class basic science department, and playing a critical role in the scientific direction of one of the most respected and renowned cancer centers in the world. Applications are encouraged from outstanding senior-level scientists in the broad field of biochemistry.

The Chair is expected to maintain a strong research program, and will have the opportunity to recruit faculty and shape a research focus for the department. Current areas of faculty research are in chromatin biology and epigenetics, stem cells and cancer, development, gene regulatory networks and intracellular signaling, translational control and structural biology. The administrative responsibilities include oversight of faculty recruitment, development, evaluation, promotion and retention; development of integrated educational programs for post-doctoral fellows and students; finance and budget; strategic planning and program development; and resource allocation, including laboratory space. The new Chair will also be important in supporting the activities of the Graduate Program in Genes & Development, which is composed of a diverse faculty and a highly successful graduate training program within the University of Texas Graduate School of Biomedical Sciences at Houston.

We are committed to providing the new Chair with major resources to support the highest level of research. MD Anderson offers unparalleled opportunities to engage in interactions with clinical, translational and basic scientists. With the newly launched Moon Shots Program at MD Anderson, the institute is making an unprecedented effort to dramatically accelerate the pace of converting scientific discoveries into clinical advances that reduce cancer deaths. The breadth and quality of biomedical science in the Texas Medical Center rivals that of any academic medical center in the world. MD Anderson is also home for the University of Texas Graduate School of Biomedical Sciences at Houston. We are looking for individuals who would excel in a challenging and rewarding leadership position, while maintaining a dynamic research program.

Interested individuals should contact us preferably via e-mail and include their curriculum vitae, names of references and a two-page narrative to address their qualifications by November 30, 2012. Send to: aburnett@mdanderson.org

MD Anderson Cancer Center is an equal opportunity employer and does not discriminate on the basis of race, color, national origin, gender, sexual orientation, age, religion, disability or veteran status except where such distinction is required by law. All positions at The University of Texas MD Anderson Cancer Center are security sensitive and subject to examination of criminal history record information. Smoke-free and drug-free facility.

Chair, Department of Biochemistry and Molecular Biology

Biochemistry and Molecular Biology
Chair Search Committee
Attn: Alice Burnett
Office of the Provost and EVP
The University of Texas
MD Anderson Cancer Center
1515 Holcombe Boulevard, Unit 1492
Houston, TX, 77030



Assistant Professor, Department of Systems Biology

The Department of Systems Biology at Harvard Medical School invites applications for a tenure-track Assistant Professor position. We seek broad and careful thinkers with strong theoretical foundations (e.g. in physics, mathematics, or computer science) who have a demonstrated commitment to tackling challenging biological or medical questions. Harvard Medical School offers a unique concentration of biomedical expertise, and the Department of Systems Biology has created a supportive and interactive environment for researchers with quantitative and theoretical skills. The successful candidate will become a member of the university-wide Ph.D. Program in Systems Biology, which has an established record of attracting extraordinary graduate students.

The deadline for applications is **November 30, 2012**. Candidates should have a Ph.D. in a relevant discipline or an MD.

To apply, please submit a statement of research interests (~ three pages), a curriculum vitae, and the names and e-mail addresses of at least three colleagues who have agreed to write letters of recommendation to <https://academicpositions.harvard.edu/postings/4336>. Once you apply, your recommenders will be prompted to submit their letters on this application portal; please make sure your recommenders submit their letters before the deadline.

Candidates should have a Ph.D. in a relevant discipline or an MD.

Applications from, or nominations of, women and minority candidates are encouraged. Harvard is an Affirmative Action/Equal Opportunity Employer.

PENNSTATE



Three Ph.D. fellowships are available within the Department of Food Science at The Pennsylvania State University beginning August 2013. Funding comes from the United States Department of Agriculture in support of the agency's focus on education within the broader area of Food and Nutrition for Health. Successful applicants would choose one or more research mentors among 16 faculty members who specialize in food safety, probiotics, food components, food structure, bioactive components, and food choice and consumer behavior (foodscience.psu.edu/directory/faculty). Fellows would also develop leadership skills through organizing a seminar series on the scientific basis of controversies and health claims in Food Science and Nutrition, and serving as mentors to students from traditionally underrepresented backgrounds who are considering graduate school.

This position is restricted to U.S. citizens due to the nature of funding. Requires at least a B.S. degree in Food Science, Microbiology, Chemistry, Engineering, Nutrition, or related field expected by May 2013. Strong preference will be given to applicants with M.S. degrees. Fellowships include a generous stipend (approximately \$24,500 per year), tuition remission, and health benefits. Applicants should apply through the Graduate School at Penn State (foodscience.psu.edu/graduateprograms/ apply) and additionally send a 1-2 page statement describing their interest in this specific program and career goals to **Dr. Edward G. Dudley, The Pennsylvania State University, Department of Food Science, 326 Food Science Building, University Park, PA 16802, dudley@psu.edu**. Deadline for applications is **December 15, 2012**.

To learn more about the Department of Food Science at Penn State, please visit our website at: <http://foodscience.psu.edu/>

Employment will require successful completion of a background check(s) in accordance with University policies. Penn State is committed to Affirmative Action, Equal Opportunity, and the diversity of its workforce.



TUM CREATE is a research programme sponsored by the National Research Foundation, Singapore. It is a joint effort of Technische Universität München (TUM) and Nanyang Technological University Singapore (NTU). It aims at developing innovative technologies and future transportation concepts in the field of electro-mobility to meet the challenging requirements of fast growing tropical mega-cities. Electric vehicles will play a major role in future traffic systems, including all of their related technologies, e.g., batteries, embedded systems, vehicle technology, and infrastructure. The individual projects and work packages are developed and performed in close collaboration with industry partners. All researchers are academically linked to one or both of the partner universities TUM and NTU. The programme supports an active exchange of students and scientists between Singapore and Munich, Germany. The research programme is run by the company TUM Create Ltd, a 100% subsidiary of TUM Asia Pte. Ltd.

TUM Create is seeking to fill the position of

Chief Executive Officer (CEO) TUM Create Ltd. in Singapore

Applicants must have a strong research background in any field related to electro-mobility, either from natural sciences or engineering. They should also have extensive experience in directing large research projects. An academic affiliation on the professorial level with either TUM or NTU is possible. The salary will commensurate with experience.

The initial appointment will be for 3 years. Eligible candidates hold a doctorate, have established a strong track record in academia and/or industry, and demonstrate pedagogical and personal aptitude, as well as substantial international experience. Family leave will be taken into consideration.

Applications accompanied by supporting documentation of professional experience should be submitted by **1st November 2012** to **Dr. Markus Wächter (markus.waechter@tum-asia.edu.sg)**.



UNIVERSITY OF OREGON

Faculty Positions in Systems Neuroscience

The **Institute of Neuroscience** (<http://uoneuro.uoregon.edu>) and the **Department of Biology** at the University of Oregon seek to fill one open-rank and one junior tenure-related faculty position in Fall 2013. We are particularly interested in candidates using the mouse to address fundamental questions in systems neuroscience, but we invite applications from researchers using Zebrafish and other model organisms, as well as those addressing questions in the development of neuronal circuitry. The successful candidate will be expected to develop a vigorous extramurally funded research program and to teach at both the graduate and undergraduate levels. Ph.D. required.

Applications accepted online for the University of Oregon NEURO SEARCH at <https://academicjobsonline.org/ajo/jobs/2113>. Applicants should submit a cover letter, a curriculum vitae including a publication list, a statement of research accomplishments and future research plans, a description of teaching experience and philosophy, and three letters of recommendation. Submission of 1-3 selected reprints is encouraged. Application materials should be uploaded by **November 15, 2012**, but the search will remain open until the positions are filled. Positions are subject to a criminal background check.

Women and minorities are encouraged to apply. The University of Oregon is an Equal Opportunity/Affirmative Action Institution committed to cultural diversity and compliance with the Americans with Disabilities Act, and supportive of the needs of dual career couples. We invite applications from qualified candidates who share our commitment to diversity.

UNCF • MERCK Science Initiative

Science Scholarships and Fellowships

The UNCF•Merck Science Initiative is an innovative approach that creates opportunities in the biological, chemical and engineering sciences for African American students throughout the country.



UNDERGRADUATE Science Research Scholarships

- Scholarships up to \$25,000
- Internship opportunities
- Mentoring and networking opportunities
- Eligibility: College juniors, science or engineering majors, 3.3 GPA

GRADUATE Science Research Dissertation Fellowships

- Fellowships up to \$53,500
- Mentoring and networking opportunities
- Eligibility: Ph.D. or equivalent degree candidates engaged in dissertation research in the biological, chemical or engineering fields

POSTDOCTORAL Science Research Fellowships

- Fellowships up to \$92,000
- Mentoring and networking opportunities
- Eligibility: Ph.D. or equivalent degree recipients in the biological, chemical or engineering fields

APPLY ON-LINE

UNCF.org/umsi
Submit by December 3, 2012

T 202 810 0331
F 202 234 0225
E uncfmerck@uncf.org



GENERAL ELIGIBILITY REQUIREMENTS:
Must be African American and a U.S. citizen or a permanent resident

Max Planck Institute of Immunobiology and Epigenetics

Max-Planck-Institut für Immunbiologie und Epigenetik



MAX-PLANCK-GESELLSCHAFT

We are looking for

Postdoctoral Position in Bioinformatics

The Max Planck Institute of Immunobiology and Epigenetics in Freiburg, Germany is offering a Postdoctoral Position in Bioinformatics in the Laboratory of Chromatin Regulation (Head: Dr. Asifa Akhtar). The Position is available for an initial two-year appointment with the possibility of extension.

The MPI in Freiburg is an international research institute at the cross-road of Southern Germany, Switzerland and France. The working language is English. State-of-the-art infrastructure and service units, including transgenesis, mass spectrometry, proteomics, flow cytometry, fly and imaging facilities are available.

Your tasks: The research focus of the Akhtar laboratory includes mechanisms underlying chromatin and epigenetic regulation. We are particularly interested in X chromosomal regulation using flies and mouse models employing multi-disciplinary approaches such as genetics, biochemistry, functional genomics as well as cell biology and structural biology.

We are looking for enthusiastic, highly-motivated, science-driven and experienced postdoctoral fellows to join our team to unravel the molecular mechanisms that regulate gene expression.

Your qualifications: Applicants should have a PhD or equivalent doctoral degree with at least 3 years of proven research experience in bioinformatics and analyses of genomewide data (ChIPseq, RNA seq). Prior experience working in Drosophila or mammalian models is highly encouraged. Candidates must have a strong publication record. Furthermore, the ability to work in a team, communication skills and experience in the supervision of graduate students are an asset.

Please submit your application, including a statement of research interests, a CV and three references to our homepage: <http://www.ie-freiburg.mpg.de/jobs>

We offer: Salaries will be according to postdoctoral fellowships of the Max Planck Society or TVöD and will commensurate with experience.

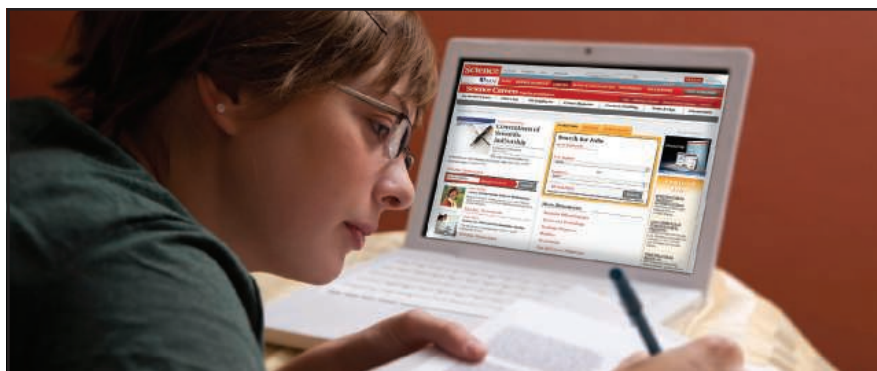
Application deadline: 30.11.2012

Our institute investigates the molecular basis of the immune response and other topics of the developmental biology, such as the origin and differentiation of the immune cells as well as the development of vertebrate embryo. Another main focus of the institute is epigenetic. This area deals with inheritable traits, which are not caused by changes in the DNA sequence.

Handicapped applicants with equal qualifications will be given preferential treatment. The Max Planck Society seeks to increase the number of women in areas, where they are underrepresented, and therefore explicitly encourages women to apply. A childcare facility is directly attached to the institute. If you would like to work in a dedicated team, please convince us now by sending us your complete application documents together with your salary expectations and your earliest possible date of joining the institute.

Max Planck Institute of Immunobiology and Epigenetics, Ms Weigold

Have we sparked your interest? Please apply online via the jobmarket at our homepage. We are looking forward to getting your complete application documents. <http://www.ie-freiburg.mpg.de/jobs>



AAAS is here – helping scientists achieve career success.

Every month, over 400,000 students and scientists visit ScienceCareers.org in search of the information, advice, and opportunities they need to take the next step in their careers.

A complete career resource, free to the public, *Science Careers* offers a suite of tools and services developed specifically for scientists. With hundreds of career development articles, webinars and downloadable booklets filled with practical advice, a community forum providing answers to career questions, and thousands of job listings in academia, government, and industry, *Science Careers* has helped countless individuals prepare themselves for successful careers.

As a AAAS member, your dues help AAAS make this service freely available to the scientific community. If you're not a member, join us. Together we can make a difference.

To learn more, visit
aaas.org/plusyou/sciencecareers



THE UNIVERSITY
OF CHICAGO

Academic Career Opportunities Position Title: Instructor-Professor Req #01475

The Department of Biochemistry and Molecular Biology at The University of Chicago invites applications for positions on its faculty at any rank. We are interested in scientists with outstanding accomplishments in and potential for fundamental research in biochemistry and/or molecular biophysics, broadly defined, and a zest for teaching. The interests of these individuals should synergize with the research in this department and other relevant biomedical programs. Generous start-up support will be provided to prime a robust research program.

Applications must include a curriculum vitae that details all publications and accomplishments, as well as plans for future research, and a teaching statement. Applications must be submitted on-line by **December 14, 2012** to academiccareers.uchicago.edu/applicants/Central?quickFind=52517. Those applicants for appointment as Assistant Professor should arrange for three letters of reference to be sent to the **Search Committee, Department of Biochemistry and Molecular Biology, 929 E. 57th Street, Room W225, Chicago IL 60637** or to BMB@bsd.uchicago.edu.

The University of Chicago is an Affirmative Action/Equal Opportunity Employer.



DREXEL UNIVERSITY
College of
Medicine

Dean Graduate School for Biomedical Sciences and Professional Studies

Drexel University College of Medicine, a leading academic medical research institution in Philadelphia and one of the premier metropolitan private research universities in the nation, seeks a scholar, scientist, educator, and innovative administrator for the position of Founding Dean of the newly established Graduate School for Biomedical Sciences and Professional Studies. The formation of the new school provides an exciting opportunity to build on the strengths of existing, vibrant, high quality programs in a student-centered environment. The key mission of this position is to provide leadership and effective management of the Biomedical, Graduate and Postgraduate Studies Programs and Professional Studies Programs in the Health Sciences of the College of Medicine. This includes quality oversight of all research-related educational and training activities and professional studies activities within the College. Applicants must hold a Ph.D. in the biomedical sciences, M.D. or equivalent degree and qualify for the rank of Professor. The successful candidate will have a proven track record in the conduct of funded biomedical research, in the training of MS, PhD, and MD-PhD students, and in the development, oversight and administration of graduate education programs. The applicant must provide evidence of a strong commitment to education and research and exhibit knowledge and understanding of what is needed in today's environment to grow, integrate and sustain high quality, research and training programs in the biomedical sciences for a diverse graduate student body. The ideal candidate will have excellent skills in building relationships, communicating effectively, and inspiring others.

Nominations and applications (including a letter of interest and curriculum vitae) should be directed, to **James Burns, PhD, Chair, Graduate School Search Committee, Drexel University College of Medicine, 245 N. 15th Street, MS 400, Philadelphia, PA 19102** or via e-mail to: apadolin@drexelmed.edu by **December 15, 2012**. Additional details can be found at www.drexelmedjobs.com. For further information about Drexel University College of Medicine, please visit www.drexelmed.edu.

Drexel University is an Equal Opportunity, Affirmative Action Employer.

Faculty Position in Bioengineering

The California Institute of Technology invites applications for a tenure-track faculty position in Bioengineering. Bioengineering research at Caltech focuses on the application of engineering principles to the design, analysis, construction, and manipulation of biological systems, and on the discovery and application of new engineering principles inspired by the properties of biological systems.

Applications are invited in any area of bioengineering research. Candidates with strong commitments to research and teaching excellence are encouraged to apply. We expect to make the appointment at the assistant professor level, but consideration will be given to exceptionally well-qualified applicants at the associate and full professor levels. Initial appointments at the assistant professor level are for four years, and are contingent upon completion of the Ph.D. degree.

The Bioengineering Option (<http://www.be.caltech.edu>) includes faculty from the Divisions of Engineering and Applied Science (<http://www.eas.caltech.edu>), Chemistry and Chemical Engineering (<http://www.cce.caltech.edu>), Biology (<http://www.biology.caltech.edu>), and Physics, Mathematics, and Astronomy (<http://www.pma.caltech.edu>).

Application Instructions: Applicants should electronically submit an application at: <http://www.be.caltech.edu/search>, including curriculum vitae; a statement of research interests; a statement of teaching interests; and up to three representative publications. Applicants should arrange to have four reference letters uploaded.

Application review will commence immediately and continue until the position is filled.



CALIFORNIA INSTITUTE OF TECHNOLOGY
*Division of Engineering and Applied Science Caltech
is an Equal-Opportunity/Affirmative-Action Employer.
Women, minorities, veterans, and disabled persons are
encouraged to apply.*



SingHealth is the largest healthcare group in Singapore, offering a complete range of multi-disciplinary and integrated medical care through our network of 2 hospitals, 5 National Specialty Centres and 9 Polyclinics. SingHealth is looking for talented individuals with potential to join us in our quest for medical excellence. If you share our vision to be a Trusted Leader in healthcare, we want to hear from you. SingHealth invites applications for the position of

SENIOR MANAGER / ASSISTANT DIRECTOR SINGHEALTH CENTRALISED INSTITUTIONAL REVIEW BOARD

Duties and Responsibilities

- Manage the Centralized Institutional Review Board (CIRB) in accordance with the department's SOPs and applicable regulatory requirements
- Oversee day to day operations of CIRB including drawing up work plans, budgets and setting KPIs
- Prepare, review and maintain SOPs to ensure compliance with applicable rules and regulations
- Initiate new ideas, develop concepts and manage all aspects of projects and assignments, ensuring effective and timely execution, and successful outcomes
- Lead, manage, guide and mentor the CIRB's team

Job Requirements

- Master's degree in Biomedical Science or related discipline
- At least 8 years' experience in IRB / research regulatory administration
- Extensive knowledge of human subjects regulations and an in-depth understanding of related regulatory, ethical and human research compliance principles
- Familiarity with ICH GCP, SGGCP and FDA regulations, especially in the area of safety reporting
- Extensive experience in clinical trials design, global and local clinical trials development processes and handling IRB accreditation standards and processes
- Good team leadership and managerial experience, with stakeholder management abilities
- Good analytical and organization skills, independent, resourceful with high emotional quotient

An attractive compensation package commensurate with experience and qualifications will be offered to the successful candidate.

Interested candidates should submit their curriculum vitae to: maria.yong@singhealth.com.sg by **15 December 2012**.

We regret that only shortlisted candidates will be notified.



Vice President

The Research Corporation for Science Advancement (RCSA) invites applications and nominations for the position of Vice President of the Corporation. The Vice President reports to the President and will carry out responsibilities of a corporate officer, working closely with the President and the Chief Financial Officer.

Founded in 1912, RCSA is the oldest foundation in the United States devoted wholly to science. It focuses on providing catalytic and opportunistic funding for high-risk scientific research and the development of academic scientists. The emphasis is on the physical sciences – chemistry, physics and astronomy—and closely related fields such as biochemistry and biophysics. RCSA's main office is located in Tucson, Arizona.

The Vice President's responsibilities will include leading the foundation's science programs and peer review process, developing relationships with external communities—colleges, universities, federal agencies, and professional societies, managing the administrative staff, and serving as RCSA leader in the absence of the President.

For more information about the Corporation, please visit www.rescorp.org. A complete search profile with information about RCSA and the desired attributes for the Vice President may be found at www.academic-search.com in the "Current Searches" Section.

NOMINATIONS AND APPLICATIONS: Review of nominations and applications will begin immediately, and nominations and expressions of interest will be welcomed until an appointment is made. The appointee is expected to take office in Fall 2013. Applications received by **November 19, 2012**, will be assured of full consideration; these should include a letter of interest, a curriculum vitae, and names of five professional references with e-mail addresses and telephone numbers. All submissions will be treated in confidence and should be sent electronically (MS Word format preferred) to: RCSAVP@academic-search.com. The search committee is assisted by: **Dr. R. Stanton Hales, Senior Consultant, Academic Search, Inc., rsh@academic-search.com • 707-545-2203**.

RCSA is an Equal Opportunity Employer.

Electrical and Systems Engineering



Tenured/Tenure-Track Faculty Positions

The Department of Electrical and Systems Engineering of the School of Engineering and Applied Science at the **University of Pennsylvania** invites applications for tenured and tenure-track faculty positions at all levels. Candidates must hold a Ph.D. in Electrical Engineering, Systems Engineering, or related area. The department seeks individuals with exceptional promise for, or proven record of, research achievement, who will take a position of international leadership in defining their field of study, and excel in undergraduate and graduate education. Leadership in cross-disciplinary and multi-disciplinary collaborations is of particular interest. We are interested in candidates in all areas that enhance our research strengths in

1. Nanodevices and nanosystems (nanophotonics, nanoelectronics, integrated devices and systems at nanoscale),
2. Circuits and computer engineering (analog and digital circuits, emerging circuit design, computer engineering, embedded systems), and
3. Information and decision systems (communications, control, signal processing, network science, markets and social systems).

Prospective candidates in all areas are strongly encouraged to address large scale societal problems in energy, transportation, health, economic and financial networks, critical infrastructure, and national security. Diversity candidates are strongly encouraged to apply. Interested persons should submit an online application at <http://www.ease.upenn.edu/faculty-positions> including curriculum vitae, statement of research and teaching interests, and the names of at least four references. Review of applications will begin on December 1, 2012.

The University of Pennsylvania is an Equal Opportunity Employer. Minorities/ Women/Individuals with Disabilities/Veterans are encouraged to apply.

POSITIONS OPEN

CHAIR

Department of Psychology
University of Texas at Arlington
Search Code: SCI092012PSY

The University of Texas (UT) at Arlington seeks an eminent scientist to lead its expanding programs in the Department of Psychology. A Ph.D. in psychological science is required, along with a proven record of excellence in research, teaching, and service. The successful candidate will have demonstrated academic leadership experience or show potential to assume such a role, as well as a clear vision for the development of the department as part of a rapidly expanding, research-oriented university. The position is offered at the rank of **FULL PROFESSOR** with tenure, with a start date as early as January 2013.

The successful candidate is expected to maintain a creative, independent, and externally funded research program, contribute to formal undergraduate and graduate teaching as necessary, engage in long-term strategic planning, and mentor junior faculty. The new chair will also play a leadership role in shaping future hiring, curriculum development, strengthening of doctoral studies, and growing the department's research portfolio. In addition, the new chair will be expected to participate in interdisciplinary collaborations that leverage emerging strengths such as cognitive neuroscience and imaging, and build bridges with regional medical institutions (University of Texas Southwestern Medical Center, University of North Texas Health Science Center, Baylor University Medical Center). We especially seek a psychologist working in the areas of health psychology, cognitive neuroscience, or related areas; however, all areas will be considered. The ideal candidate will be one who has a broad understanding and appreciation of research areas across psychological science, and who is capable of fostering interdisciplinary initiatives and collaborations. The ability to promote and support an applied perspective of psychology to enhance the natural and interrelated relationships between science and practice is also highly desirable. *Women and minorities are strongly encouraged to apply.*

UT Arlington, situated on a cloistered, urban campus in the Dallas-Ft. Worth Metroplex, is a vital and diverse academic community of over 33,000 undergraduate and graduate students working together with faculty committed to outstanding teaching, research, and scholarship. The Psychology Department is home to M.S. and Ph.D. programs focusing on several areas of psychology (neuroscience, health, developmental, social/personality, industrial/organizational), as well as a vibrant undergraduate program. Additional information can be found at **website:** <http://www.uta.edu/psychology/index.htm>.

Completed applications should consist of curriculum vitae, a statement of research interests and goals, a statement of teaching interests and evidence of teaching quality, a statement of leadership experience or potential, and the names and contact information of at least five references.

Review of applications will begin November 9, 2012, but applications will be accepted until the position is filled. *A criminal background check will be conducted on finalists.* Inquiries about this position may be directed to **Dr. James Grover** (e-mail grover@uta.edu, telephone: 817-272-9495), or **Ms. Amy Osborn** (e-mail osborn@uta.edu, telephone: 817-272-3444). We prefer applications in Adobe PDF format submitted electronically to e-mail: osborn@uta.edu. Print applications can be mailed to: **Psychology Chair Search Committee, 501 S. Nedderman Dr., Box 19047, Arlington, TX 76019.**

UT Arlington is an Affirmative Action/Equal Opportunity Employer. *Women, minorities, veterans, and individuals with disabilities are encouraged to apply. The use of tobacco products is prohibited on UT Arlington properties.*

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Science Careers

From the journal *Science*



Vice President of Research and Collections

For more than 110 years, the Denver Museum of Nature & Science has served as a catalyst for curiosity by conducting scientific research, preserving precious artifacts and specimens, displaying the wonders of our natural world, and offering lifelong science education. Last year, we welcomed 1.4 million visitors onsite and at offsite Museum programs. Exhibitions at the Museum offer enriching experiences that bring science, nature, and culture to life. Seeing the "real thing" is what visitors remember long after their visit has ended—everything from real collections to real discoveries, like the Museum's largest-ever fossil excavation, The Snowmastodon Project, which is providing new scientific information about the evolution of life in the Rockies and beyond.

DMNS has a rare opportunity for a visionary scientist to provide leadership for the Museum to ensure quality scientific research, appropriate stewardship of collections, and to support dynamic educational programming and exhibition content. Reporting to the CEO, the Vice President of Research & Collections is the top scientific executive in the institution. The VP will provide forward thinking leadership which will drive focus, efficiency, and innovation, to ensure a robust scientific core that allows the Museum to be positioned for the future. The VP will play an active role in fundraising for museum projects and fostering productive and long-lasting donor relationships. Additionally, the VP leads the Museum's public trust responsibilities by assuring legal accountability and long term preservation of collections. This individual will oversee an operating plan that incorporates annual and long-term goals, objectives, and strategies for the branches of Earth & Space Sciences, Zoology & Health Sciences, Anthropology, and Preservation & Documentary Resources.

Ideal Candidate: Accomplishing this critically important task will require a PhD-holding scientist with a nationally recognized current or past research program. The Vice President position requires a leader of exceptional vision, experience, and personal integrity with a passion for education and great science. This individual will bring at least 10 years of progressive administrative management and leadership experience, solid fundraising skills, media relations experience, a background in informal science education and an understanding of the legal and ethical issues surrounding collections. Additionally, the ideal candidate has a distinguished record of excellence and experience in leading and managing teams to produce results consistent with organizational mission and vision for the future.

If you are interested in applying for this position or would like to nominate a candidate, please send your current CV along with a letter of interest to VPRCD@dmns.org. Initial applicant review will begin on **October 31, 2012**.

The Denver Museum of Nature & Science is an Equal Opportunity Employer. The Museum is dedicated to the goal of building a culturally diverse staff committed to serving the needs of all our visitors.

Abramson Family Cancer Research Institute Faculty Position

The Abramson Family Cancer Research Institute at the Perelman School of Medicine at the University of Pennsylvania seeks candidates for a Full or Associate Professor position in the tenure track. Rank will be commensurate with experience. Applicants must have an M.D. or M.D./Ph.D. or equivalent degree. Board certification is preferred.

This is a joint recruitment of the AFCRI and the Division of Hematology-Oncology in the Department of Medicine. We are seeking candidates who are maintaining an independent, extramurally funded research program on mechanisms of hematologic malignancies and/or solid tumor pathogenesis and progression. Current strengths within the Penn Cancer Community include investigation into the basic mechanisms of cancer metabolism, the tumor microenvironment, epigenetics, responses to stress and/or DNA damage, immune cell responses to pancreatic, breast, melanoma, and other malignancies, tumor dormancy, metastasis, and developmental therapeutics.

The faculty appointment will be in the Department of Medicine and the successful candidate will hold an important leadership position within the AFCRI, as well as play a critical role in the recruitment of future faculty to the Hematology-Oncology Division led by Lynn Schuchter, M.D. Additional information can be found at <http://www.pennmedicine.org/hematology-oncology/>.

The Abramson Family Cancer Research Institute (AFCRI) was established in 1997 through an initial \$100 million gift from Leonard and Madlyn Abramson. Since that time, the Abramson Foundation has committed an additional \$25.5 million towards the AFCRI, which serves as a forum for integrating cancer research, education, and patient care in partnership with the Abramson Cancer Center at the University of Pennsylvania led by Chi Van Dang, M.D., Ph.D. The AFCRI seeks to promote basic science excellence, while transforming scientific breakthroughs into innovative treatments as quickly as possible. As Scientific Director of the AFCRI, M. Celeste Simon, Ph.D. leads an accomplished faculty who hold a variety of leadership roles across undergraduate, graduate, medical, and postdoctoral training at the University of Pennsylvania. Additional information about the AFCRI can be found at www.afcri.upenn.edu.

Dr. Celeste Simon is the Chair of the Search Committee. Position will remain open until filled. We seek candidates who embrace and reflect diversity in the broadest sense.

The University of Pennsylvania is an equal opportunity, affirmative action employer.

Apply for this position online at: http://www.med.upenn.edu/apps/faculty_adlindex.php/g323/d3049.



Perelman
School of Medicine
UNIVERSITY OF PENNSYLVANIA



UNIVERSITY AT ALBANY
State University of New York

7 Positions at The RNA Institute at the University at Albany

ADVANCED COMPUTATIONAL FACILITY LAB MANAGER: supervise High Performance Compute (HPC) facility; provide team leadership; conduct and support RNA computational research in structure/function relationships; learn and adapt technologies, provide analysis, integrate, innovate and deliver results supporting human disease research. Vacancy announcement in its entirety and apply online at: <http://albany.interviewexchange.com/jobofferdetails.jsp?JOBID=34949>

ADVANCED INSTRUMENTATION FACILITY LAB MANAGER: supervise and support high end instrumentation for RNA research; provide team leadership in collaborations to support researchers with high end instrumentation, methods and analysis; discover, learn and adapt technologies, provide analysis, integrate, innovate and deliver results supporting human disease research. Vacancy announcement in its entirety and apply online at <http://albany.interviewexchange.com/jobofferdetails.jsp?JOBID=34918>

Qualifications: Ph.D. from accredited U.S. university or equivalent from a foreign university and 3+ years related experience with 1 year prior supervisory experience in a team-oriented, collaborative, multi-project environment. Preferred: productive postdoctoral training and demonstrated ability to obtain independent, extramural funding.

ADVANCED INSTRUMENTATION FACILITY ASSISTANT LAB MANAGER: assist with oversight and management of shared Advanced Instrumentation Facility and Radiochemical Facility; maintain lab infrastructure related to the operation of RNA specialized lab; make lab as usable and flexible as possible. Qualifications: B.S. (Master's preferred) in biology/chemistry/molecular biology/microbiology and 2 years postgraduate in academic/industrial biochemical labs. Vacancy announcement in its entirety and apply online at <http://albany.interviewexchange.com/jobofferdetails.jsp?JOBID=34948>

POSTDOCTORAL ASSOCIATES – BIOCHEMISTRY/STRUCTURAL CELL & MOLECULAR BIOLOGY: 4 positions in various research projects and collaborations conducting research on RNA, RNA interactions, and RNA imaging reporting to Director, Paul F. Agris. Supervise and support instrumentation users. Vacancy announcement in its entirety and apply on-line at <http://albany.interviewexchange.com/jobofferdetails.jsp?JOBID=34893>

Competitive salary and benefits. Successful candidates work in The RNA Institute (<http://www.albany.edu/rna>) in state-of-the-art labs in the Life Sciences Research Building (<http://www.albany.edu/lifesciences>), uptown campus, Albany, NY. The Institute creates an outstanding collaborative research environment for ~40 regional investigators.

All applicants must possess excellent organizational, verbal, written and interpersonal skills. In applying candidates address their ability to work with and instruct a culturally diverse population. Application review begins immediately and continues until positions are filled.

The University at Albany is an EEO/AA/IRCA/ADA employer.

POSITIONS OPEN



RESEARCH ASSISTANT PROFESSOR SUNY Upstate Medical University

The Department of Urology at SUNY Upstate Medical University, College of Medicine, is seeking a candidate for the position of Research Assistant Professor to conduct basic research in the field of urologic oncology. Candidates should have a Ph.D. and/or M.D. and postdoctoral research experience. Ample opportunities for collaboration with basic and clinical scientists interested in cancer research are available. Candidates will be expected to seek extramural funding, but an attractive startup package will be provided.

Please forward a cover letter, curriculum vitae, summary of research interests, and names of three references to **Barbara McConnell** at e-mail: mcconneb@upstate.edu.

DIRECTOR of The Laboratory of Infectious Diseases Faculty Position in Microbiology and Immunology

The Department of Microbiology and Immunology at the University of South Alabama invites applications for a position as Director of the Laboratory of Infectious Diseases, a new 25,000 square foot BSL-2/BSL-3 facility currently under construction. The selected individual will hold the rank of Associate Professor or Professor in the Department of Microbiology and Immunology. We seek an outstanding virologist investigating basic mechanisms of pathogenicity, although candidates with research interests in the areas of bacterial or fungal pathogenic mechanisms, as well as the immunology of infectious diseases, are encouraged to apply. Applicants must hold a Ph.D., M.D., or M.D.-Ph.D. degree; have a strong record of research productivity; current extramural funding; and expertise in BSL-3 laboratory operations and research compliance. The successful candidate will be expected to participate in teaching graduate and medical students. The Department has an active research group focused on intracellular, select agent pathogens and opportunities for interdisciplinary interactions with the Center for Lung Biology and the Mitchell Cancer Institute are available. For additional information about the Department, visit [website: http://www.usahealthsystem.com/microbiology](http://www.usahealthsystem.com/microbiology).

Please submit curriculum vitae, a description of current and future research plans, and the names and contact information of three references to: **David O. Wood, Ph.D., Department of Microbiology and Immunology, 5851 USA Drive North, Room 2102, Mobile, Alabama 36688-0002**. Application materials are best sent electronically to e-mail: pcouling@southalabama.edu. Applications will be reviewed beginning October 1, 2012 and received until the position is filled.

The University of South Alabama is an Equal Opportunity/Equal Access Employer.

TENURE-TRACK FACULTY CLUSTER HIRE UConn Marine Sciences

The Department of Marine Sciences in the College of Liberal Arts and Sciences at the University of Connecticut invites applications for three tenure-track faculty positions for a new initiative on Climate and Human Alteration of Coastal Ecosystems (CHACE). We seek to fill positions in the areas of: (1) coupled atmosphere-ocean modeling, (2) geochemistry/paleoceanography, and (3) marine ecology/evolutionary biology. One position will be at the **ASSISTANT PROFESSOR** level and the other two at **OPEN RANK** (preference will be given to pre-tenure candidates). For details on the positions, qualifications, and application instructions, see [website: http://www.marinesciences.uconn.edu/jobs.php](http://www.marinesciences.uconn.edu/jobs.php). The University of Connecticut is an Equal Employment Opportunity/Affirmative Action Employer. (Search numbers 2013226, 2013227, and 2013228).

POSITIONS OPEN



HARVARD MEDICAL SCHOOL
ASSISTANT PROFESSOR IN THE DEPARTMENT OF CELL BIOLOGY

ASSISTANT PROFESSOR in The Department of Cell Biology Harvard Medical School

The Department of Cell Biology at Harvard Medical School invites applications for a Tenure-Track Assistant Professor faculty position in the area of metabolism. Candidates must have a Ph.D., M.D., or equivalent degrees and a record of outstanding research productivity. Areas of interest include the regulation of metabolism at the biochemical, cellular or organismal level; the integration between metabolism and other cellular or developmental processes; or metabolism alterations and their role in disease. The Department offers ample laboratory space and a highly competitive startup package. Please visit our [website: http://cellbio.med.harvard.edu/](http://cellbio.med.harvard.edu/) for more information about the department.

Please submit curriculum vitae, a two-page summary of past accomplishments and research plans, and three references letters by November 10, 2012 to [website: https://academicpositions.harvard.edu/postings/4327](https://academicpositions.harvard.edu/postings/4327).

Harvard Medical School is an Equal Opportunity/Affirmative Action Employer.

TWO ASSISTANT PROFESSOR POSITIONS Department of Chemistry and Biochemistry Florida State University

The Department of Chemistry and Biochemistry at Florida State University (FSU) invites applications for two tenure-track positions at the Assistant Professor level beginning fall 2013. Successful candidates are expected to develop an internationally recognized, externally supported research program and to teach undergraduate and graduate courses. Applicants should have a Ph.D. in Chemistry or related field.

One position is in advanced measurement technologies, especially those developing/utilizing bioanalytical techniques with an emphasis on sensors, separations, optical and magnetic resonance spectroscopy, and mass spectrometry. Applications should be sent to e-mail: expt_search@chem.fsu.edu. Review of applicants will begin October 15 and continue until the position is filled.

The second position is in theoretical/computational chemistry. The Department is particularly interested in individuals with research interests in developing and applying emerging theoretical/computational methods to tackle chemical or biochemical problems related to human health, energy, or materials. Applications should be sent to e-mail: theory_search@chem.fsu.edu. Review of applicants will begin November 9 and continue until the position is filled.

To be considered for these positions, send curriculum vitae, research plan, a statement of teaching philosophy and interests, and three letters of recommendation electronically or by mail to: **Faculty Search, Department of Chemistry and Biochemistry, Florida State University, 95 Chieftan Way, Tallahassee, FL 32306-4390**. Florida State University is a Public Records Agency and an Equal Opportunity/Access/Affirmative Action Employer; minority candidates and women are especially encouraged to apply.

A POSTDOCTORAL FELLOW or JUNIOR FACULTY position is available at the Center of Excellence for Novel Approaches to Neurotherapeutics at Mount Sinai School of Medicine (New York, New York) to study mechanisms associated with Alzheimer's disease neuropathology. The candidate should be an expert in animal models of neurodegeneration, in particular transgenic mouse models of neurodegeneration and neuropathology. The candidate would be involved in drug discovery studies aimed at the identification of novel agents for the prevention of amyloid neuropathology in Alzheimer's disease. The candidate should be proficient in genomic techniques. For those who wish to apply, please contact **Amanda Bilski** (e-mail: amanda.e.bilski@mssm.edu), Program Coordinator.

POSITIONS OPEN



The Morehouse School of Medicine Department of Physiology is soliciting applications from excellent candidates in the field of physiology to join our faculty at the **ASSOCIATE** or **FULL PROFESSOR** level. We are seeking to expand upon our current strengths in gastrointestinal physiology, cardiovascular physiology, reproductive endocrinology, and renal physiology. A Ph.D. or M.D. in a related field and independent university research experience is required. The ideal applicant should have current extramural support for independent research projects and a strong publication record. The successful candidate should also have excellent teaching skills that will contribute to the department's educational programs. Please address inquiries to:

**Gianluca Tosini, Ph.D.,
Interim Chair, Department of Physiology
Morehouse School of Medicine
720 Westview Drive, MEB 350
Atlanta GA 30310
Phone: 404-752-1680
E-mail: gtosini@msm.edu**

Applicants should submit their curriculum vitae, at least three references for letters of recommendation, and statements of research and teaching interests to the Chairperson. Final determination of candidacy will be based upon a successful interview. *Minorities, women, persons with disabilities, and Veterans are encouraged to apply at the Morehouse School of Medicine where there is a strong commitment to Equal Employment Opportunity.*

ECOLOGIST

The Department of Biological Sciences at Bowling Green State University (BGSU) seeks applicants for an **ASSISTANT PROFESSOR** level, tenure-track Ecologist who addresses questions at large spatial scales. We welcome candidates who focus on landscape ecology (terrestrial or aquatic), spatial analysis of ecological processes, implications of changing land use on ecological communities and ecosystems, or other spatial dynamics. Applicants with postdoctoral experience and research expertise that complements existing strengths in population and conservation ecology are preferred. Successful candidates are expected to develop a highly productive, externally funded research program, as well as contribute to the teaching missions of our Ph.D.-M.S. program (90 students) and undergraduate program, which includes a Specialization in Ecology and Conservation Biology. To apply, electronically submit cover letter, curriculum vitae, statements of research plans and teaching philosophy/experience, representative publications, and three reference letters to e-mail: dmclean@bgsu.edu or by mail to: **Ecologist Search Committee, Department of Biological Sciences, BGSU, Bowling Green, OH 43403-0208** by November 16, 2012. A background check is required for employment. Questions? Contact **Karen Root** at e-mail: kvroot@bgsu.edu. Further information about our department can be found at [website: http://www.bgsu.edu/departments/biology](http://www.bgsu.edu/departments/biology).

BGSU is an Affirmative Action/Equal Opportunity Employer/Educator and encourages applications from women, minorities, veterans, and persons with disabilities.

BAYLOR COLLEGE OF MEDICINE Houston, TX

Baylor College of Medicine seeks an **ASSISTANT PROFESSOR** in Houston, Texas. Requirements: Ph.D. in Molecular Biology, three plus years experience with viral propagation in human liver chimeric mice. Travel Requirement: 10% domestic travel to attend conferences. Apply online at [website: http://www.medschooljobs.org](http://www.medschooljobs.org). Use job code 224833EH.

Baylor College of Medicine is an Equal Opportunity, Equal Access/Affirmative Action Employer.

PRIZES

Call for Nominations: Scolnick Prize in Neuroscience

The McGovern Institute for Brain Research is accepting nominations for the 10th annual Edward M. Scolnick Prize in Neuroscience. The Prize recognizes an outstanding discovery or significant advance in the field of neuroscience. The prize is \$100,000. The recipient presents a public lecture at MIT, hosted by the McGovern Institute and followed by a dinner in Spring 2013.

Nomination Deadline: December 15, 2012

Nomination procedures: Candidates for the award must be nominated by individuals affiliated with universities, hospitals, medical schools, or research institutes, with a background in neuroscience. Self-nomination is not permitted.

Each nomination should include: • A biosketch or CV of the nominee • A letter of nomination with a summary and analysis of the major contributions of the nominee to the field of neuroscience • Up to two representative reprints will be accepted.

Selection Procedure: Members of the selection committee and faculty affiliated with MIT are not eligible. Announcement of the award recipient will be made in February 2013. Recipient must attend all events to be awarded the prize.

Past Scolnick Prize Recipients:

2004: Dr. Masakazu Konishi, California Institute of Technology
2005: Dr. Judith L. Rapoport, National Institutes of Mental Health/NIH
2006: Dr. Michael E. Greenberg, Children's Hospital/HMS
2007: Dr. David Julius, University of California, San Francisco
2008: Dr. Michael Davis, Emory University School of Medicine, Atlanta
2009: Dr. Jeremy Nathans, Johns Hopkins University
2010: Drs. Lily and Yuh-Nung Jan, UCSF
2011: Dr. Bruce McEwen, The Rockefeller University
2012: Dr. Roger Nicoll, University of California, San Francisco

Send nomination packet to:

Attn: Scolnick Prize Nomination
 McGovern Institute for Brain Research
 Massachusetts Institute of Technology
 77 Massachusetts Avenue 46-3160
 Cambridge, MA 02139; or gwolf@mit.edu

For more information: <http://mcgovern.mit.edu>

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discover what's new. Learn about the latest research, challenges in translational medicine and future areas of promise. Help to advance the science.

call for posters
 submit your abstract by October 19
www.worldstemcellsummit.com



POSITIONS OPEN

ASSISTANT PROFESSOR The Redox Biology Program at The Medical College of Wisconsin

The Redox Biology Program, directed by Neil Hogg, Ph.D., at the Medical College of Wisconsin is seeking two full time Ph.D., M.D., or foreign equivalent faculty at the level of Assistant Professor. We are seeking highly motivated individuals to develop an independent program in the field of oxidative biology and/or redox signaling. The primary activity of the successful applicants will be academic research and the ability to develop strong collaborative programs with faculty members within the various departments at MCW. Applicants are required to demonstrate a level of independence and expertise in their field of research, along with at least two years of experience of postdoctoral training in some of these areas of research. The successful candidates must be able to perform both self-directed and guided work and possess excellent collaborative skills with researchers. Qualified applicants are invited to apply by November 30, 2012. Send a complete curriculum vitae, three or more letters of recommendation and a personal statement of research interest to: Elisabeth Witkowski, Department of Biophysics, Medical College of Wisconsin, 8701 Watertown Plank Rd, Milwaukee, WI, 53226. E-mail: ewitkowski@mcw.edu. Compensation is dependent upon qualifications. The Medical College of Wisconsin provides a generous package of fringe benefits. Review of the applications will commence December 1, 2012 and continue until the positions are filled. *The Medical College of Wisconsin is an Affirmative Action/Equal Opportunity Employer.*

POSTDOCTORAL FELLOWS

The Ansari ALS Center for Stem Cell and Regeneration Research at Johns Hopkins University is seeking two postdoctoral fellows in the following fields: (1) to study stem cell transplantation from IPS cells and fetally derived neural stem cells in models of neurodegenerative disease with an emphasis on motor neuron diseases (ALS). The fellowship will use stem cell methodologies to both investigate disease pathogenesis as well as therapeutics. Preference will be given to candidates whose research is related to the pathogenesis of neurodegenerative disorders and/or stem cell biology. (2) to study molecular mechanisms of muscle atrophy in chronic denervation. Experience in regeneration studies in the peripheral nervous system is desirable but not required. Experience in muscle biology and bioinformatics is preferable.

Interested candidates should have a Ph.D. or M.D. degree, and a demonstrated capacity to perform creative and original research. Applicants interested in the stem cell position should send their resumes electronically to Dr. Nicholas J. Maragakis at e-mail: nmaragakis@jhmi.edu. Applicants interested in the muscle position should send their resumes electronically to Dr. Ahmet Hoke at e-mail: ahoke@jhmi.edu.

TENURE-TRACK FACULTY POSITIONS in Biological Timing

The Department of Biological Sciences at North Dakota State University (NDSU) seeks to fill two ASSISTANT PROFESSOR positions to begin fall 2013. We welcome applicants studying ecological and evolutionary consequences of seasonal timing. We will consider applicants studying timing at all levels of inquiry, from those studying genetic, molecular, and cellular mechanisms as well as those studying community, landscape, and ecosystem-level functions. The Department offers competitive start-up packages and a supportive environment for faculty growth. See website: <http://www.ndsu.edu/biology/> for a complete description and online application. Review of applications will begin October 15, 2012. *NDSU is an Equal Opportunity/Affirmative Action Employer and an ADVANCE and Carnegie Very High Research Activity Institution. This position is exempt from North Dakota Veterans' Preference requirements.*

POSITIONS OPEN

TENURE-TRACK POSITIONS The University of Texas Southwestern Medical Center

ASSISTANT PROFESSORS: The Department of Physiology invites outstanding scientists with Ph.D., M.D., or equivalent degrees to apply for tenure-track assistant professor positions. Candidates who use innovative optical, mechanical, electrical, molecular biological or computational methods with important applications to physiological systems, ranging from individual genes and proteins to cells and organs are encouraged to apply. However, the scientific excellence of the candidates is more important than the specific area of research.

These positions are part of the continuing growth of the Department at one of the country's leading academic medical centers and will be supported by significant laboratory space on our new campus, competitive salaries, and exceptional startup packages. The University of Texas Southwestern (UT Southwestern) Medical Center is the scientific home to five Nobel Prize Laureates and many members of the National Academy of Sciences and Institute of Medicine. UT Southwestern conducts more than 3,500 research projects annually totaling more than \$417 million.

Applicants should submit curriculum vitae, a brief statement of research plans, and arrange to have three letters of reference sent to: James Stull, Ph.D., c/o Ronald Doris, Department of Physiology, The University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390-9040.

UT Southwestern strongly encourages applications from women, minorities, and people with physical challenges. UT Southwestern is an Equal Opportunity/Affirmative Action Employer.

SYSTEMATIC BOTANIST (Research Botanist)

The Department of Botany seeks a systematic botanist for a full-time research position, initially as a four-year term appointment. Candidates should have demonstrated expertise that emphasizes innovative as well as conventional application of systematic techniques/theory, utilizing modern methods of comparative morphology and tools such as molecular phylogenetics. Candidates should also have expertise in additional fields, such as biogeography, biodiversity and conservation, floristics, informatics, or theoretical systematics. Candidates with a recognized research program on the systematics of lichens, ferns, marine algae, or a major angiosperm group such as Asteraceae, Fabaceae, Melastomataceae, and Rubiaceae, in which the U.S. National Herbarium has strong holdings, may be given preference. The position will be filled at the GS-12 level with a starting salary of \$74,872. *U.S. citizenship is required.* Applicants must have demonstrated ability to establish an externally funded research program, and to conduct fieldwork and/or collection building. See announcement number 13A-JW-297816-DEU-NMNH at websites: <http://www.sih.si.edu> or <http://www.usajobs.gov> for details about the application process for this position. To learn more about the Smithsonian's Botany Department, see website: <http://botany.si.edu/>. Applications must be received online by November 15, 2012 and must reference the announcement number. Applicants will be notified by e-mail when their applications are received. *The Smithsonian Institution is an Equal Opportunity Employer.*

FACULTY POSITIONS - MEDICAL SCHOOL

The Saint James School of Medicine, an international medical school (website: <http://www.sjsm.org>), invites applications from candidates with teaching and/or research experience in any of the basic medical sciences for its Caribbean campuses. Faculty positions are currently available in Anatomy, Physiology, Pathology, Microbiology, and Pharmacology. Applicants must be M.D., D.O., and/or Ph.D..

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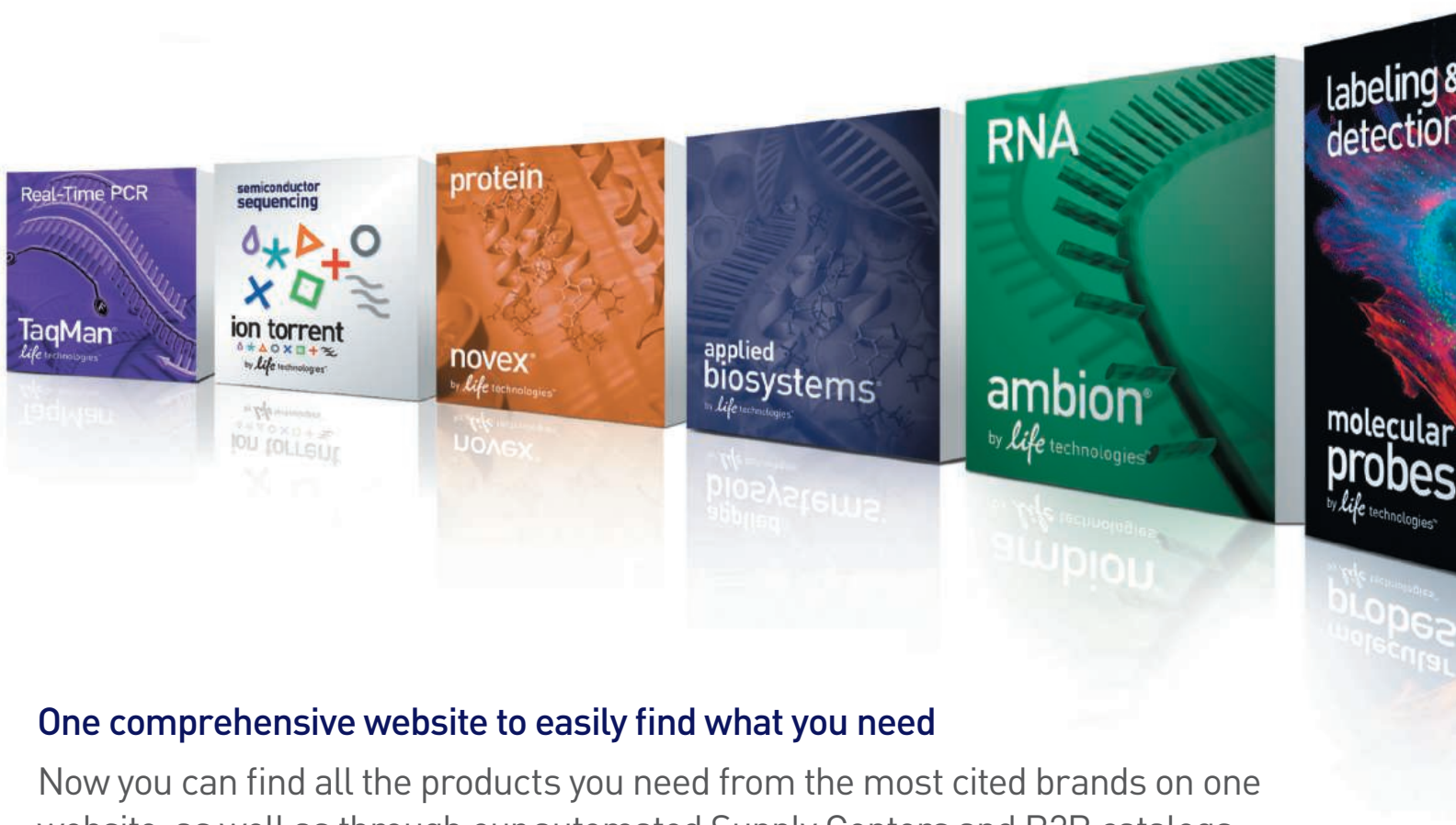
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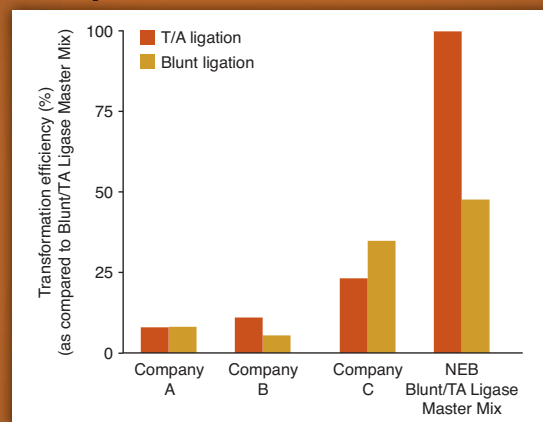
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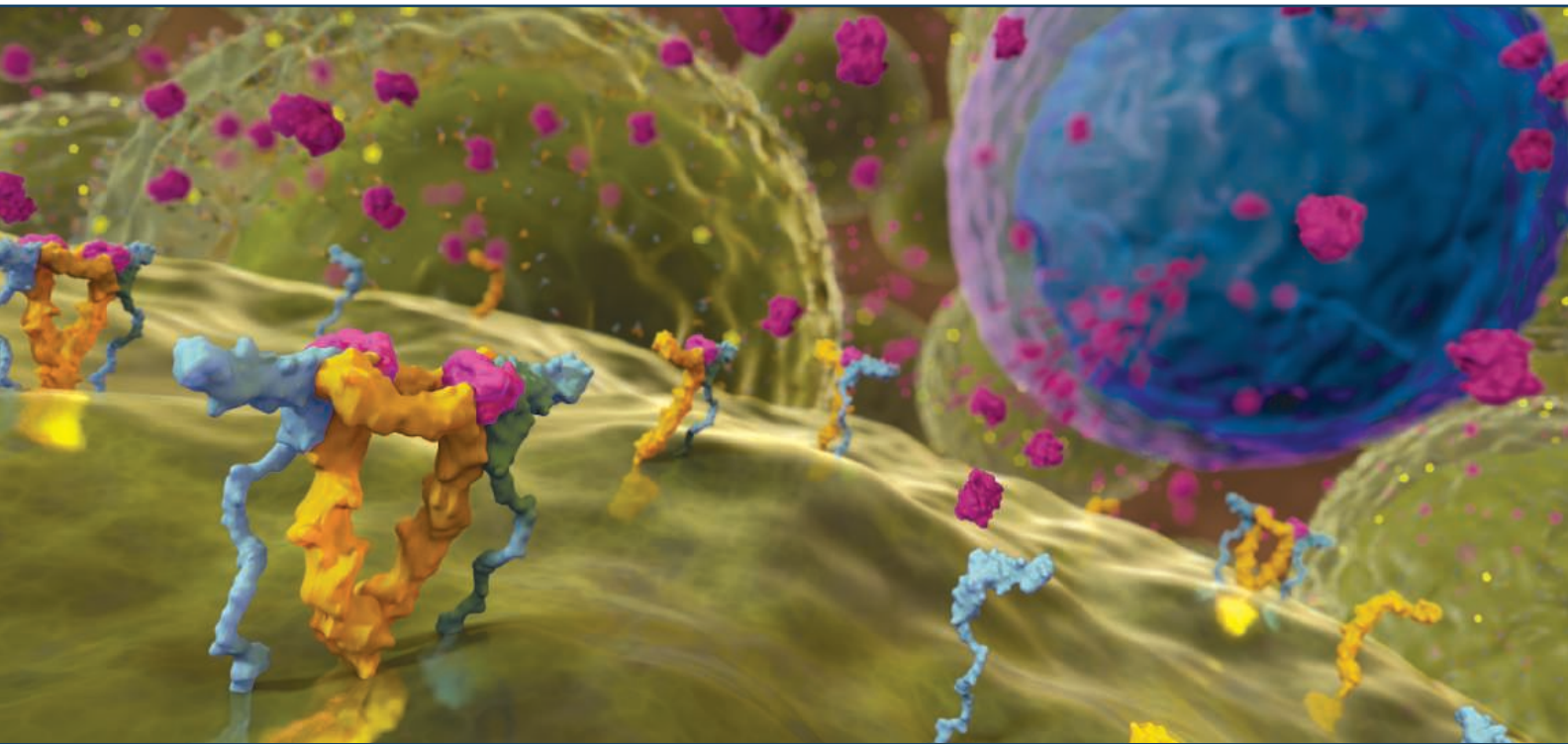
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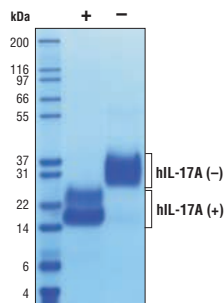
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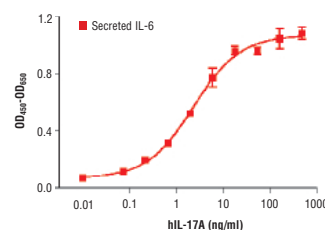
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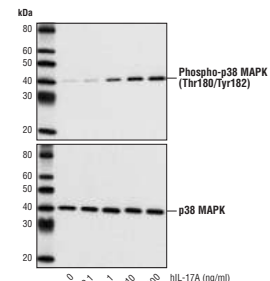
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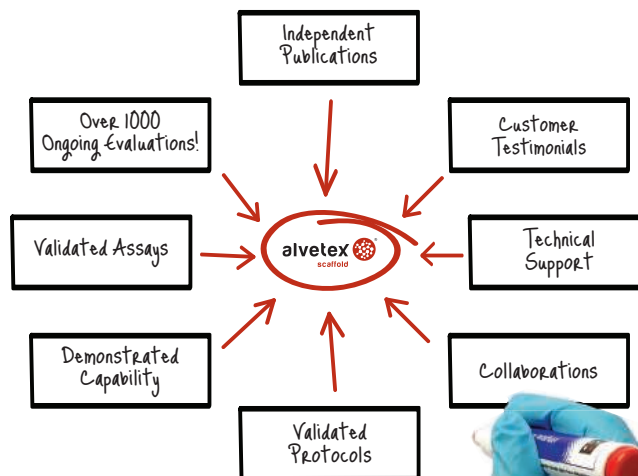
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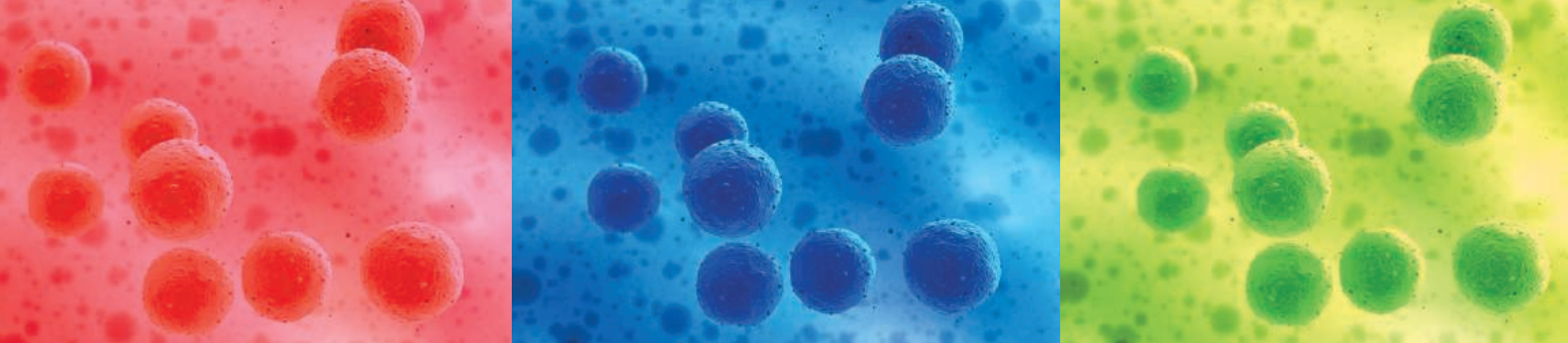
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WEBINAR

What Cytometry Can Do For You

The Pros and Cons of Image and Flow Cytometry

October 30, 2012

12 noon ET; 9 a.m. PT; 4 p.m. UK; 5 p.m. CET

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WEBINAR VIEWERS WILL:

- Learn how experts are using cytometry techniques to advance their research
- Discover the value of combining image cytometry, including HCA, with traditional flow cytometry
- Gain a better understanding of the pros and cons of both image and flow cytometry, and how these techniques might be applied to their own research
- Have their questions answered live by our in-studio panel!

FEATURED PARTICIPANTS

Paul Gokhale, Ph.D.
University of Sheffield
Sheffield, UK

Bill Telford, Ph.D.
National Cancer Institute, NIH
Bethesda, MD

Liz Roquemore, Ph.D.
GE Healthcare
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